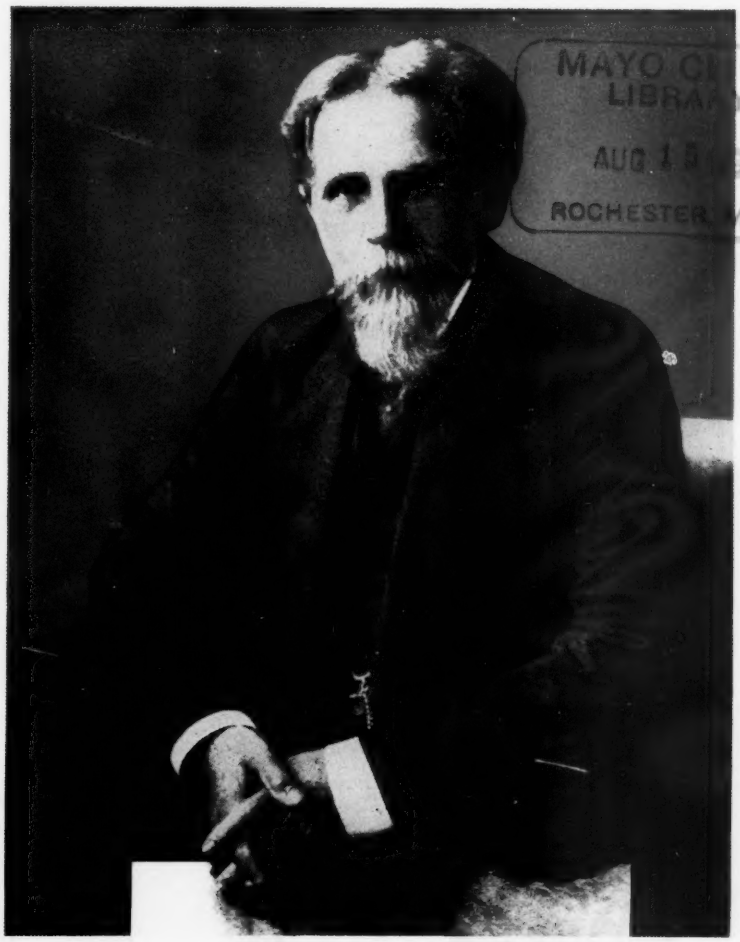
DIABETES

The Journal of the American Diabetes Association



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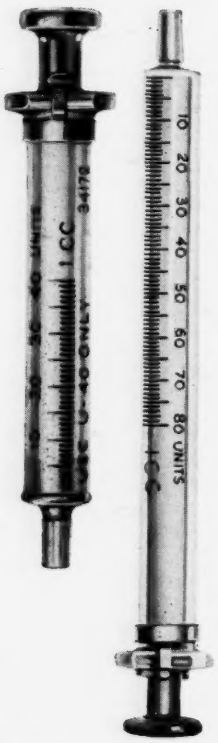

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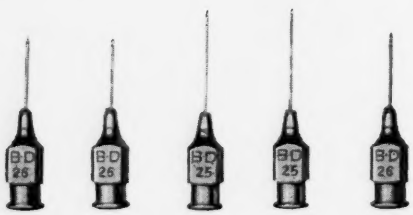

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The Responses of the Smaller Blood Vessels and the Serum Proteins in Pregnant Diabetic Subjects

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Diabetes mellitus through its disturbed metabolism and hormonal imbalance has a profound effect upon the course of pregnancy. The deviations from normal pregnancy which are encountered most frequently include a high incidence of hydramnios, toxemia, congenital fetal abnormalities and perinatal death.[1, 2] It is well established that, when the disturbed metabolism is being controlled meticulously with insulin and diet, fetal mortality falls dramatically, though not to normal.[3, 4] It is not known how a poor control of diabetes during pregnancy will produce harmful effects on host and fetus. Suggestions have been made relating it to imbalance of female sex hormone production[5] and to increased adrenocortical activity accompanying the difficult glucose regulation during pregnancy.[6]

Venning and Browne[7, 8] demonstrated increased urinary corticoid excretion in normal pregnancy. Gemzell[9] and Robinson et al.[10] found that the amount of 17-hydroxycorticosteroids in the blood increased progressively during the course of pregnancy and fell to the normal levels a few weeks after delivery. Although few such studies have been performed in diabetic pregnancy, the figures by Gray[11] indicate that a similar rise occurs in the urinary excretion of glucocorticoids during diabetic pregnancy, although it is not quantitatively different from excretion during nondiabetic pregnancy.

The current literature also indicates an aggravating influence of pregnancy upon the manifestations of vascular disease in diabetes. Lawrence[12] reported two cases of severe hemorrhagic retinopathy which first developed during pregnancy and reversed completely following delivery. Sherrill[13] presented a case in which "myriads of round discrete aneurysms" suddenly appeared in the retinas of a pregnant diabetic and disappeared quickly after pregnancy was terminated and control of diabetes was established. Becker[14] added two similar cases and one of very early retinopathy which advanced rapidly during pregnancy and regressed spontaneously to its original level following delivery. White[15] also suggested that retinopathy may be aggravated during pregnancy. However, she found that progression of existing retinopathy was evident in only five diabetic cases during pregnancy in contrast to no progression in sixty-three cases, or 93 per cent, of those with retinopathy. The pathogenesis of the late diabetic complications is far from clear. It has been pointed out that vascular disease in diabetes may be a consequence of prolonged influence of insulin-antagonists, especially the corticosteroids, which may be produced, among other things, by fluctuating blood sugar levels.[16, 17]

On account of this information it was considered important to study the changes occurring in the smaller blood vessels during pregnancy in diabetics. The retina would have been most favorable as a field for direct microscopic observations, but the lack of magnification, as provided with the ophthalmoscope, makes observations of capillaries and the small branches of arterioles and venules impossible. This disadvantage can be overcome by using the stereoscopic dissecting microscope on the easily accessible bulbar conjunctiva. Much evidence points toward the generalized nature of the microangiopathy in diabetics[18] and observations made upon the conjunctival vascular bed suggest that degenerative lesions are taking place in this tissue though with less characteristic features (i.e., no microaneurysm) than in the retina.[19] It is likely, however, that the greater intensity and specificity of vascular pathology in the retina may be explained by the high functional need for an optimal circulation (high local metabolism) and the particular architecture (end-capillaries with definite basement membrane[20]) of the vascular bed in the retina. Since the conjunctival vessels can be studied with a much higher magnification than those of the retina, *precursory* changes might be visualized which are not observable in the retina. It was found that the smaller blood vessels, especially those of young diabetics, responded intermittently with pathologic reversible changes.[21] The vessel changes included arteriolar constriction and venular distention, associated with intra-arteriolar aggregation of red cells, slowing of linear velocity of blood cells, and exudation. The observations suggested that these vasomotor and flow changes in the

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small blood vessels over the years were involved in the mechanism leading to the degenerative changes in the vascular bed. In this respect it is worth emphasizing that Bloch[22] observed the consistency of the arterial blood in the brain, intestine, uterus, bladder, skeletal muscle, etc., to appear identical in the arterioles of the bulbar conjunctiva in Rhesus monkeys and cats. This implies that some changes, comparable to those observed in the conjunctival vessels, take place elsewhere in the organism.

Recently Landesman and co-workers studied the responses of the bulbar conjunctival vascular bed during normal[23] and toxemic pregnancy.[24] They observed during the course of normal pregnancy a progressive constriction of the arterioles "with a reduction in blood flow," most prominent in the third trimester. Blood flow in the venules in the second trimester appeared "granular" over the entire vascular bed reaching a maximum in the last month, during labor, and for the initial days postpartum. The blood flow in the capillary bed progressively diminished, and the number of vessels visualized was significantly reduced during the last months of pregnancy. In toxemias of pregnancy a further progression of the conjunctival vascular changes took place, relative to those seen in normal pregnancy. Changes in the vascular bed of the conjunctiva appeared earlier and were more advanced than those of the retina. Various degrees of toxemia existed in sixteen pregnant diabetics studied. They found in this group that the spasm seen in the arterioles of the conjunctiva paralleled a similar finding in the retina.[24] Odell, Aragon and Pottinger[25] studied likewise the conjunctival vessels during normal and abnormal pregnancy. This investigation was mostly concerned with the extent of the intravascular erythrocyte aggregation, referred to as "sludge." They suggested from their investigation that a causal relationship existed between changes in the plasma protein pattern and the presence and extent of intravascular erythrocyte aggregation; both of these changes were more pronounced during abnormal pregnancy. A relationship between absolute or relative increases in globulins and/or fibrinogen as opposed to the albumin in plasma and the degree of intravascular erythrocyte aggregation has also been suggested to be present in diabetes mellitus.[26, 27] Mack,[28] Coryell and associates[29] as well as others[30, 31] studied the changing distribution of the blood plasma proteins during normal pregnancy. All these investigators found that the plasma albumin and γ-globulin progressively fell to the time of delivery and that the α_1-, α_2-, β-globulin and fibrinogen rose at the same time. After delivery these protein fractions returned toward the level existing before pregnancy. As a result of the above changes the albumin/globulin ratio declined continuously during pregnancy and returned toward normal some weeks after delivery. These protein changes are not specific to pregnancy but have been found also to occur in other conditions, involving tissue proliferation and tissue destruction as during infections. Studies of the conjunctival vascular changes during pregnancy indicate that 1) spasm of arterioles, 2) ischemia of the capillary bed, 3) granularity of blood flow and 4) slowing of blood flow are maximum at the time of delivery as are the protein changes.

It has been well established now that changes take place also in the plasma protein distribution in diabetics with and without vascular disease.[32–34] The alterations consist of a decrease of the albumin and an increase of the α_2-globulin, in the β-lipoprotein and in the α_2-glycoprotein fractions. The increase in the β-lipoproteins and of the carbohydrate-containing globulins has been shown to be related partly to uncontrolled hyperglycemia and glycosuria and partly to the duration of the disease and the extent of vascular degeneration.[34, 35] The accumulation of lipids and mucopolysaccharides in the vascular lesions of the diabetics and the possible relationship between changes in lipoproteins to atherosclerosis make these data interesting.

Because pregnant diabetics are likely to show pronounced protein changes, and vascular responses accompanying a progression of their vascular disease, a study was initiated to evaluate the vascular and intravascular responses of the conjunctival vascular bed and the serum protein changes serially in a group of diabetics during pregnancy. This report surveys the nature of these changes.

METHODS AND MATERIALS

The vascular bed of the lateral bulbar conjunctivae was observed with a stereobinocular microscope of the Grenough type (Leitz). Normally the observations were made with a magnification of 48x or 64x but for limited detail inspection a magnification as great as 144x may be used. A further detailed description of this method is given by Bloch.[36] The conjunctival vessels were photographed by a specially developed technic.[37] The linear velocity of the blood cells was measured in representative arterioles, capillaries and venules by timing with a stop watch the passage of the red cells along a vessel. The length of the vessel was measured by an ocular micrometer.

Particular attention has been paid in this work to

reversible response changes in the caliber of the small vessels and in the consistency of the circulating blood. Figure 1 indicates in diagram some of the more important conjunctival vascular changes observed during pregnancy. Dynamic alterations in the caliber of the minute vessels were evaluated from direct measurements and from the ratio between the diameter of the arterioles and the accompanying larger venules at various sites. The various changes were graded in arbitrary units from normal (Grade 0) to Grade 3.

Arteriolar Changes and Grading. The normal terminal arterioles measure generally between twenty to fifty micra in diameter. Grade 1 constriction of the arterioles indicates a general narrowing of the arterioles with increased vasomotion. The diameter of the arterioles measures between fifteen to thirty micra. The vasomotion observed consists of slight rhythmic changes in the diameter. Grade 2 constriction of the arterioles indicates a more pronounced narrowing of the arterioles occasionally interrupted by slight bulb formation. Grade 3 constriction indicates a spasm-like condition of the arterioles. The arterioles measure less than twelve micra, and most often only one or two may be found in the whole conjunctiva. At times, when the arterioles are markedly constricted, the true capillaries may be emptied of blood. It was found repeatedly that practically all true capillaries could disappear from the conjunctiva, the tissue of which became pale and ischemic. The blood flow was then shunted from the terminal arterioles to the venules by "preferential capillaries." These channels have recently been described in the bulbar conjunctiva of healthy subjects by Grafflin[38] and Meigham[39] who questioned their functional significance.

Capillary Changes and Grading. Another change of the capillaries is a tortuous elongation of the venous end (figure 1). Grade 1 indicates that a few capillaries show elongated angular tortuosities. Grade 2 indicates that 10 to 50 per cent of the capillaries show this characteristic and Grade 3 that 50 to 100 per cent of the capillaries show tortuosities.

Venular Changes and Grading. In many diabetics the venules become intermittently distended. This change was often observed with little or no arteriolar constriction and has been graded by measurements of the arteriolar/venular diameter ratio. Normally the A/V ratio is 1:3-1:2. Grade 1 distention indicates an A/V ratio of 1:5-1:4, Grade 2 an A/V ratio of 1:7-1:6, and Grade 3 an A/V ratio of even less.

Intravascular Changes and Grading. Intravascular erythrocyte aggregation was graded according to its presence in the venules (Grade 1), in venules and capillaries (Grade 2), or in arterioles, venules and capillaries (Grade 3). The value of this grading system has been assessed previously.[27]

It should be mentioned that in conjunctival vessels containing aqueous humor, the erythrocytes can appear aggregated, i.e., a column of clear fluid (aqueous humor) may be stratified with erythrocytes. The recognition of this possibility, especially in the region of the corneoscleral limbus, prevents such misinterpretation (figure 2a).

Perivascular Changes and Grading. Perivascular changes could be seen as edema, localized or diffuse "hyaline" infiltration and hemorrhages or pigment from old hemorrhages in the conjunctival tissue. Mild edema (Grade 1) is observable only microscopically and is indicated by slight swelling and gray color of the tissue, and by difficulty in focusing the microscope on deeply-lying structures. More advanced edema (Grade 2) may be seen macroscopically. Microscopically the tissue becomes more milky-white and appears diffusely homogeneous. If the edema partly or completely persists, the originally edematous area becomes more pearly-white and only few details can be made out (possible sign of atrophy). This stage has been designated as "hyaline infiltration" (Grade 3). In addition, localized and slightly elevated deposits with or without enclosed yellowish material may be seen.

Serum proteins were fractionated by paper electrophoresis, using a modification of the horizontal open strip method by Grassman and Hannig.[40] The paper used was Schleicher and Schuell 2043A, and the buffer a veronal-acetate, *p*H 8.6, with an ionic strength 0.1. The run lasted for fifteen hours and was carried out at 4°C. with 4.9 volts per cm. of free strip. The strips were afterwards stained for protein with Amido Black 10B, for fat with Sudan Black B and for carbohydrate with the McManus technic (periodic acid and fuchsin sulfate). The electrophoretic curves for the serum components were determined on a densitometer (Photovolt Model No. 525). The strips stained for protein were made translucent with an α-bromo-naphthalene-mineral oil mixture. Areas under the curves were then determined with a planimeter. Double determinations were made routinely on proteins, lipoproteins and glycoproteins.

In an unselected group of thirty diabetic and thirty normal pregnant women conjunctival examinations were made on the average at semimonthly intervals during their gravidity. Most of these observations were made

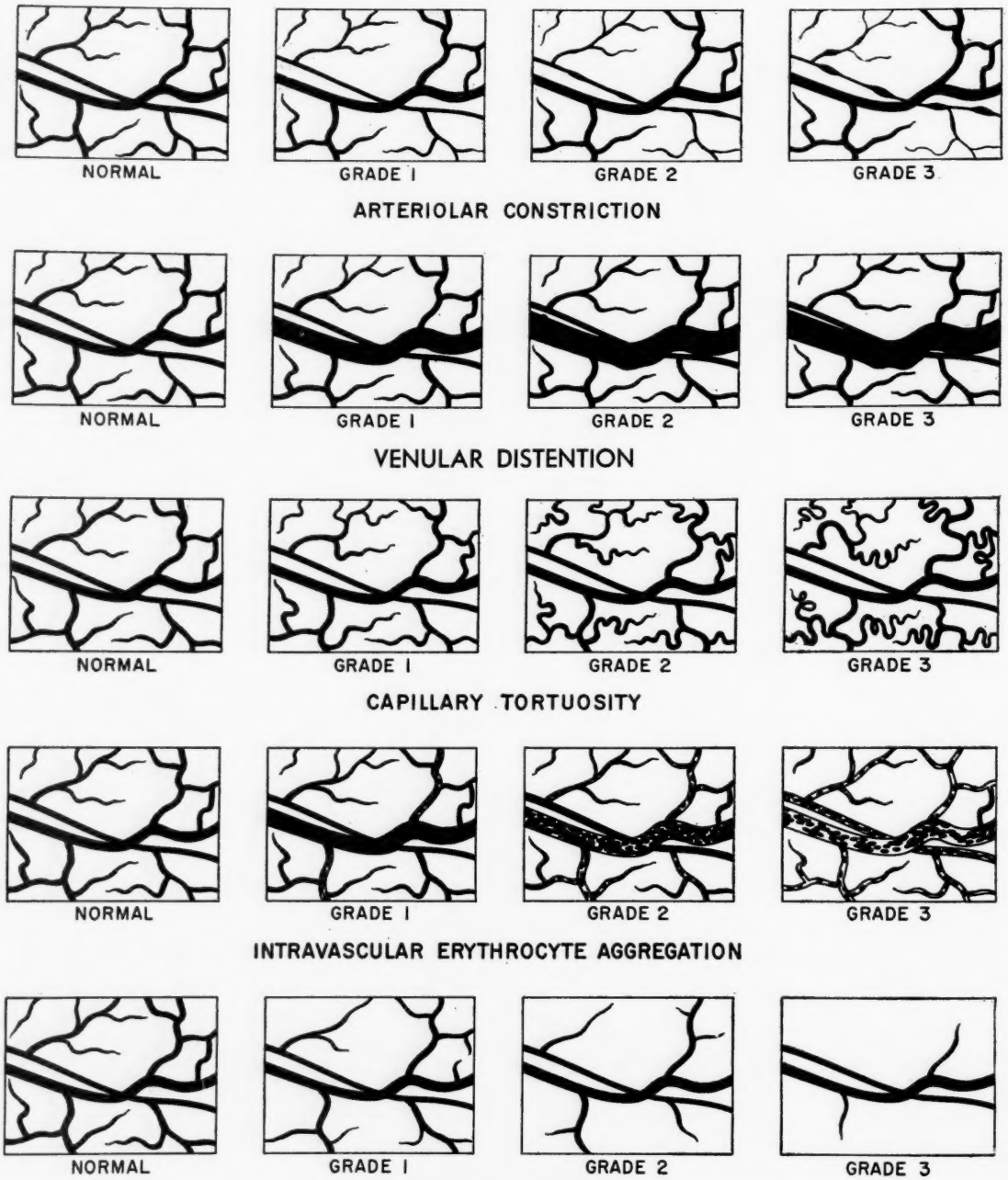

FIG. 1. Vascular changes in the bulbar conjunctiva.

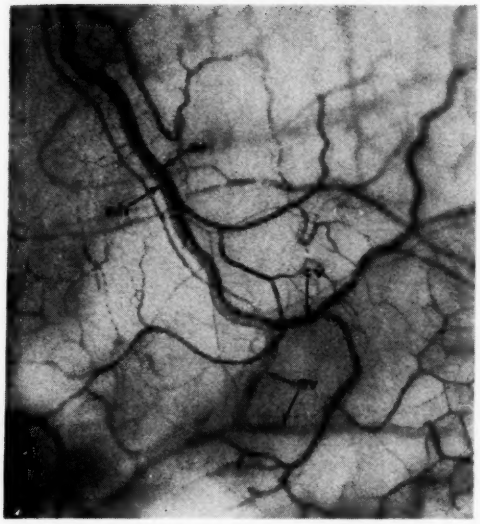

FIG. 2a. Aqueous vein (av) joined by a conjunctival venule (cv). Note the maintenance of separate identities of the aqueous humor (ah) and the blood cells (er).

between the twelfth and the thirty-third week of pregnancy. The study of the vessels took place in the morning between 10:00 a.m. and 12:00 noon and was included as part of the weekly ambulatory examination and control of the diabetic pregnancy cases by the internist and the obstetrician. To omit bias in the study all conjunctival examinations were carried out by the same observer who, other than the presence or absence of diabetes, knew no details of the medical history. Paper electrophoretic serum protein analyses were made serially in eight of these diabetics and two of the normal pregnancy cases simultaneously with conjunctival observations. Further comparative examinations have since been continued in another fifteen diabetic cases, the results of which will be reported later.

The pertinent data on all subjects are shown in table 1. All the diabetics received insulin. Their duration of diabetes varied from one to twenty-eight years, with an average of 9.4 years. In fourteen cases (47 per cent) the onset of the disease occurred under the age of fifteen years. Therefore, this series is probably unique on account of the relatively large number of patients in whom the onset occurred in childhood. Among the thirty diabetic cases, five showed diabetic retinopathy, four of whom had retinitis proliferans. Roentgen-ray studies of the leg and pelvic vessels showed evidence of calcification in nine. Three patients showed persistent proteinuria (50 mg. or more in the twenty-four-hour specimen

TABLE 1

Pertinent data on subjects under examination

	Diabetics	Normal
Number of subjects	30	30
Number of pregnancies	30	30
Age at examination		
Age range	20-37 years	21-41 years
Age average	25.8 years	29.3 years
Onset of diabetes		
Age range	1.6-25.0 years	
Age average	16.4 years	
Duration of diabetes		
Duration range	1.0-28.0 years	
Duration mean±s.d.	9.4±6.8 years	
Number of primiparae	10	7

measured with Cargille's modified Kingsbury-Clark standards for albumin). Another six cases showed proteinuria (50 mg. or above) intermittently during pregnancy. Five of these patients had simultaneously bacteriuria and pyuria. None of the cases showed the classical Kimmelstiel-Wilson syndrome (hypertension, edema, retinopathy and albuminuria, etc.). All but two patients with mild toxemia showed normal blood pressure during the whole pregnancy, i.e., a systolic blood pressure below 140 mm. Hg. and a diastolic blood pressure below 90 mm. Hg. Female sex hormone therapy was administered according to the schedule of White[5] in all but one of the diabetic subjects.

RESULTS

Table 2 illustrates the over-all result derived from the microscopic observations of the conjunctival vascular bed during diabetic and nondiabetic pregnancy. During both diabetic and normal pregnancy progressive changes took place which tended to reach a maximum at the time of delivery and to return to the prepregnant level approximately four weeks post partum. In normal pregnancy cases the alterations in the vascular bed appeared during the second trimester as "granularity" with a slowing down of the rate of flow of cells in the capillaries and in the smaller venules. In about 50 per cent of the cases the intravascular erythrocyte aggregates also appeared during the third trimester in the terminal arterioles and the larger venules, making their flow discernibly segmented. These aggregates occasionally were rigid enough to occlude the terminal arterioles for a second before they were squeezed through the capillaries. In approximately 50 per cent of the cases the arterioles showed Grade 1 constriction and increased vasomotion which was particularly prominent directly prior to delivery. The conjunctival tissue became pro-

TABLE 2

Conjunctival vascular changes in diabetic pregnancy as compared to normal pregnancy

	Arteriolar constriction		Venular distention		Capillary ischemia		Capillary tortuosity		Erythrocyte aggregation		Decreased arteriolar rate of blood cells		Decrease in the A/V ratio		Conjunctival exudation edema	
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N
1st trimester	0-2	0	0-2	0	0-2	0	1-3	0-1	1-3	0-1	0-2	0	0-3	0	0-2	0
2nd trimester	0-2	0-1	1-3	0	0-2	0	1-3	0-1	2-3	1-2	1-3	0-1	1-3	0-1	0-2	0
3rd trimester	1-3	0-2	0-2	0-1	1-3	1-2	1-3	0-1	2-3+	1-3	1-3	0-2	2-3	0-1	1-2	0-1
Post partum	0-2	0	0-2	0	0-2	0	1-3	0-1	1-3	0-1	0-2	0	0-3	0	0-2	0

D = diabetic
N = normal

gressively paler and many capillaries were emptied of blood. The flow of blood may have bypassed the capillary network through "preferential capillaries." The length or form of the capillaries did not alter during pregnancy. In the third trimester the larger venules in approximately 10 per cent of the cases showed slight distention, but the arteriolar/venular ratio did not exceed 1:5-1:4. At times slight microscopic edema of the conjunctival tissue is present a few weeks prior to delivery. Progressive alterations, accompanying these vascular changes, took place in the serum protein distribution. The amount of total protein decreased slightly together with the packed cell volume, probably due to hydremia of pregnancy. The albumin fractions fell and the globulin fractions increased, leading to a decrease in the albumin/globulin ratio. The uneventful progression of the alterations, following the pattern indicated, was characteristic of the normal pregnancy.

In sharp contrast to this was the fluctuating character of more pronounced changes in the vascular bed and in the serum proteins which took place in diabetics. The fluctuations in the vascular responses and in the serum proteins may have been due not so much to the presence of pregnancy per se but to some factor inherent in the diabetes itself, since similar vascular and serum protein alterations have been observed to occur intermittently in nonpregnant diabetics. From table 2 it can be seen that changes similar to those observed late in normal pregnancy were already present in the first trimester of many diabetic pregnancies. The degree of the vascular and serum protein responses during the first two trimesters in the pregnant diabetics as in nonpregnant diabetics appeared to depend on factors such as the degree of control of diabetes, the duration of diabetes and the extent and degree of vascular disease already present. In addition, a similar or accentuated trend was found in diabetics to exhibit progressive vascular and serum protein changes in the latter part of the second or the entire third trimester of pregnancy. The degree of changes, their possible reversibility following pregnancy, their relationship to complicating factors during pregnancy and to the outcome of pregnancy in the diabetics are described and exemplified.

1. *Arteriolar Constriction.* During normal pregnancy a slight degree of arteriolar constriction (Grade 1) could be observed in about 50 per cent of the cases during the latter part of the second trimester and particularly during the third trimester (figures 2b and 3). In diabetics the arteriolar constriction in the latter part of pregnancy occurred in a higher percentage (66 per cent) and to a greater intensity, sometimes starting early in the second trimester (figure 4). In addition to the greater tendency to progressive arteriolar constriction late in pregnancy, eight diabetic cases showed various degrees of arteriolar constriction intermittently during first and second trimester. Three cases with mild toxemia exhibited arteriolar constriction of Grade 2 from about the twenty-second week. Since this degree of arteriolar constriction has been observed also in apparently uncomplicated pregnancy cases in diabetics, this finding has but little clinical significance.

Case Report. S. C., No. 34,359. Diabetes mellitus of eleven years' duration without diabetic retinopathy. This thirty-one-year-old para I, gravida V, was first seen by the clinic in her twelfth week of pregnancy. Blood pressure was normal and there was no albuminuria present. No arteriolar constriction of her conjunctival vessels was found. In the eighteenth week the patient showed slight albuminuria which disappeared after one week and reappeared during the twenty-sixth week; the blood pressure was constantly normal. There was a trace of edema from the twentieth week on. At this time the arterioles were markedly constricted (Grade 3) and difficult to visualize. The blood flow contained erythrocyte aggregates which intermittently occluded the terminal arterioles for two to four seconds. In the twenty-fifth week the arterioles were still found to be maximally constricted (Grade 3), the capillary bed was ischemic

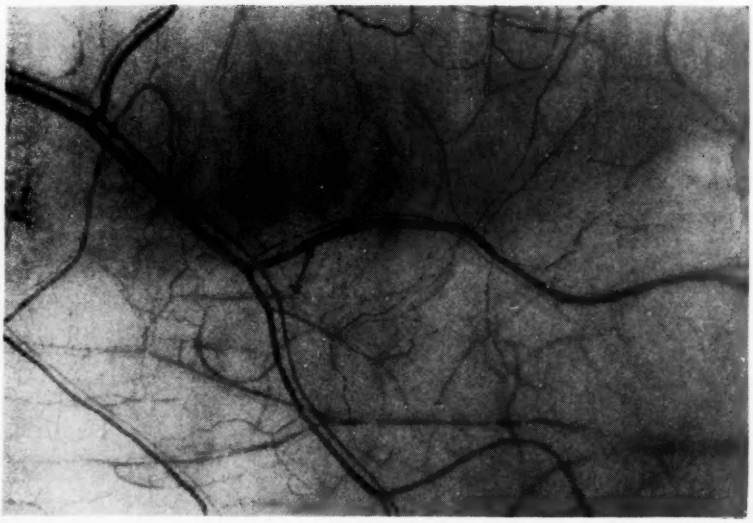

FIG. 2b. Case E. D. Normal pregnancy in second trimester.

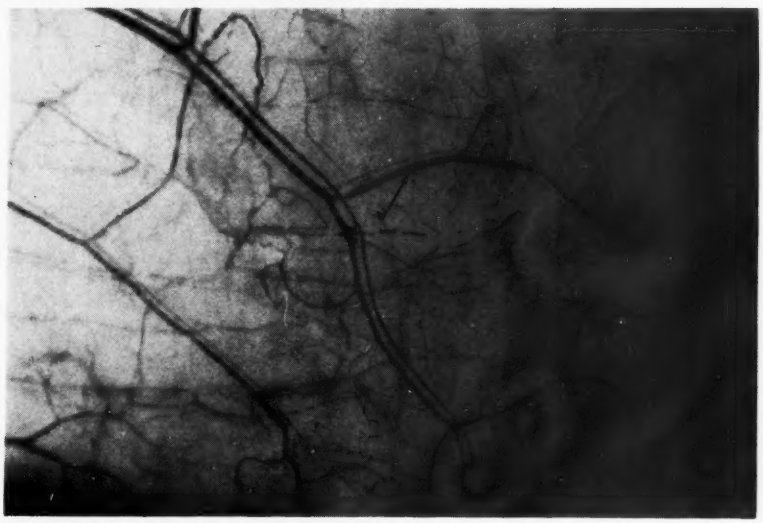

FIG. 3. Case E. D. (same case as in figure 2). Normal pregnancy late in third trimester. Note the arteriolar constriction (Grade 1) and aggregation of erythrocytes in the capillaries and venules.

and the venules narrowed. Edema of the conjunctival tissue was present. This maximum vasoconstriction remained during the following fourteen weeks (figure 5). In the twenty-ninth week the patient delivered a macerated anencephalic monster.

2. *Venular Distention.* The venules become slightly distended only very occasionally late in normal pregnancy. On the other hand, in diabetics the venules were often found to be distended, tortuous and congested during the entire pregnancy. Twenty-three (77 per cent) of the diabetics of this series showed, at one time or another, venular distention with arteriolar/venular diameter ratios approximately between 1:8-1:4. Significant variations in the degree of distention occurred in the same patients over a period of time. This distention usually progressed or reversed slowly, but occasionally in the course of a week relatively pronounced changes took place. By absolute measurements the diameter of the same venule might change from 30 micra to 100 micra. Associated with the development of marked distention, the larger venules often showed bulges, such as fusiform dilatations or larger sacculations. Swellings of this kind were observed in seven cases. In eight cases progressive distention of the venules was observed in an apparently orderly sequence of events to be accompanied by a marked decrease in linear rate of blood flow, evidence of progressive hemo-

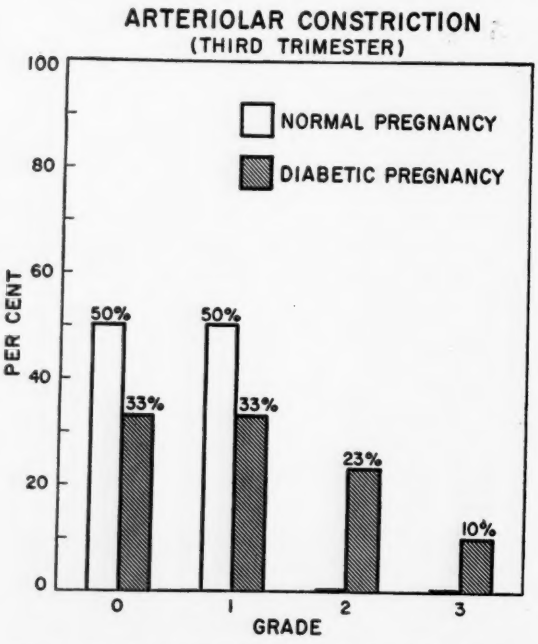

FIG. 4. Distribution of arteriolar constriction in third trimester of pregnancy.

seen to be surrounded by an opaque hazy coating resembling exudate. These changes appeared to be most prominent in the second and early part of the third trimester. In the latter part of pregnancy the venules tended to attenuate as was most often observed during nondiabetic pregnancies. The venular distention might be accompanied by a slight arteriolar constriction but, when the arteriolar constriction became maximum (Grade 3), the venules tended also to become attenuated. It appeared that, when the arterioles had a monophasic response (mainly progressive arteriolar constriction), the venules showed a diphasic response (mainly distention followed by constriction).

Case Report. W. T., No. 9,910. Diabetes mellitus of twenty-three years' duration with bilateral retinitis proliferans. This thirty-three-year-old primipara was first seen at the clinic in the fourteenth week of pregnancy. She had no albuminuria, but had bacteriuria and pyuria. Blood pressure and blood nonprotein nitrogen were normal. The conjunctival arterioles were not constricted. The arteriolar blood flow was slow and segmented and in 50 per cent of the terminal arterioles red cell aggregates plugged the tips of terminal arterioles for one to two seconds. The venules were distended and the A/V ratio measured 1:6 (figure 6). In the twenty-first week she still had asymptomatic pyuria, no albuminuria and normal blood pressure. The conjunctival arterioles were now constricted (Grade

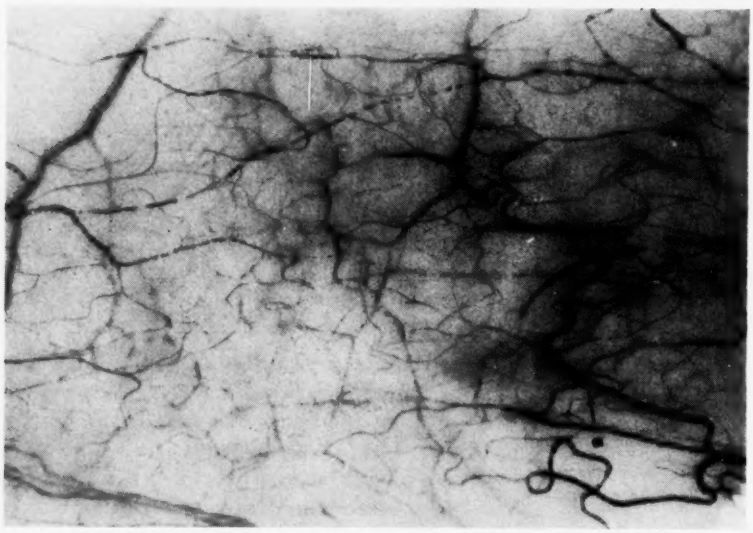

FIG. 5. Diabetic pregnancy during third trimester. (a) Arteriolar constriction (Grade 3). (v) Venule. Generalized Grade 3 aggregation of red cells.

concentration of the passing blood and the appearance of edema in the perivascular tissue. In another three cases the disappearance of venular distention was observed to coincide with an increase in the rate of blood flow and the disappearance of edema. In four cases large engorged venules with very slowly flowing blood were

2), the capillaries mostly closed, the conjunctival tissue ischemic, and the venules attenuated with an A/V ratio of 1:1, (figure 7). There was marked microscopic edema of the conjunctival tissue. In the twenty-fifth week the patient had slight albuminuria, normal blood pressure, pyuria and the same conjunctival vascular appearance. In the twenty-eighth week she delivered nonviable twins. At examination post partum the arterioles were

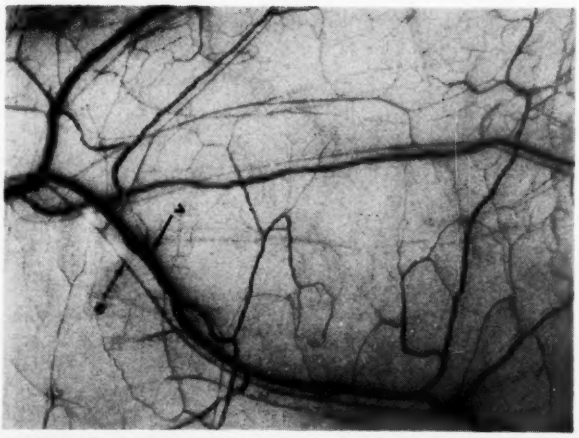

FIG. 6. Case W. T. Diabetic pregnancy in second trimester. (v) Distended venule with fusiform dilatations, (a) slightly constricted arteriole.

FIG. 7. Case W. T. (same case as in figure 6). Diabetic pregnancy late in third trimester. (a) Arteriolar constriction (Grade 2). (v) Attenuated venule. Note the ischemia of the tissue.

not constricted, the venules slightly distended with an A/V ratio of 1:5.

3. *Capillary Tortuosity.* In the normal pregnancy cases, Grade 0-1 nonprogressive capillary tortuosity could be seen. Multiparae did not have more tortuous capillaries than primiparae. In diabetics, tortuosity of the capillaries was found in all cases. The degree of capillary change varied from case to case. The distribution of the grade of capillary tortuosity, based on later observations in diabetic pregnancy, were 52 per cent with Grade 3, 42 per cent with Grade 2, and 6 per cent with Grade 1 tortuosity. In the majority of the cases no evidence of progression or regression of the changes was present.

These cases, however, showed initially pronounced elongation of the venous part of the capillaries so that any progression would be very difficult to detect. In three cases in whom the capillary abnormalities were at the beginning minimal, a definite progression of the capillary elongation during pregnancy was present which did not regress following delivery. This is exemplified in figure 8 (a and b) from the following diabetic case.

Case Report. M. W., No. 34,573. Diabetes mellitus of three years' duration. This twenty-one-year-old para II, gravida II developed hydramnios (+2) in the thirtieth week, edema from the thirty-second week on and slight rise of blood pressure (140/95) in the thirty-third week of pregnancy. Patient suffered from symptomless pyuria during pregnancy. Her diabetes was difficult to control. In the thirty-fifth week a normal infant with weight of 2,700 gm. was delivered by cesarean section. From the twenty-eighth week slight arteriolar constriction of Grade 1 was present which did not proceed. The venules were slightly distended and showed fusiform dilatations. Microscopic edema was present in the conjunctival tissue from the twenty-eighth week. Figure 8a shows a picture of the conjunctival capillaries taken in the twenty-first week and figure 8b is the same field photographed four months post partum. It shows the progression of capillary tortuosity.

4. *Edema/Hyaline Infiltration.* Microscopic evidence of edema was seen in many diabetic pregnancy cases with and without vascular distention. It was apparent, however, that edema could be formed as an immediate result of venular distention. Depending on the duration and the severity of the presumably exudative process,

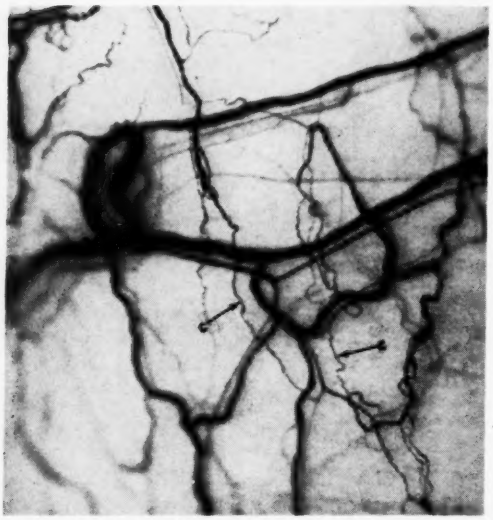

FIG. 8a. Case M. W. Diabetic pregnancy during second trimester. (c) Tortuous capillaries.

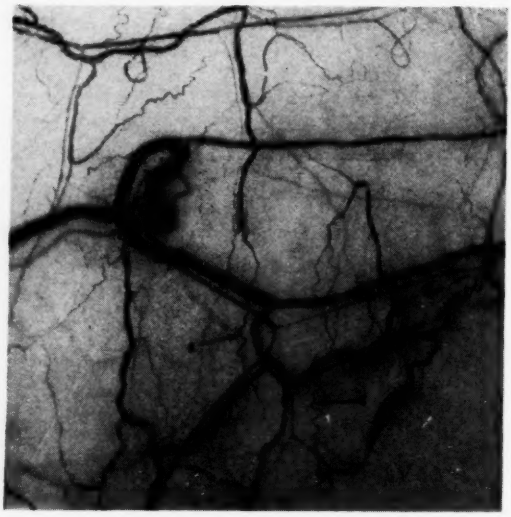

FIG. 8b. Case M. W. (same case as in figure 8a). Four months post partum. Compare capillaries (c). Note the progression of capillary tortuosity.

the perivascular tissue might change and show various degrees of abnormality. The complete transition from edema to "hyaline" infiltration was observed in three cases during the course of pregnancy.

Case Report. B. J., No. 2,474. Diabetes Mellitus. This twenty-two-year-old primipara had suffered from diabetes for nine years and showed no retinopathy or nephropathy. Patient was first examined at the clinic in the eighth week of pregnancy, at which time no ankle edema was present but the conjunctiva showed microscopic (Grade 2) edema. No hyaline infiltration. The conjunctival venules were slightly distended (A/V 1:5-1:4). Patient continued to show marked conjunctival microscopic edema during the whole pregnancy. From the twenty-seventh week the arterioles showed Grade 1 constriction and the venules were further distended (A/V 1:6) and showed a few fusiform dilatations. Thrombosis formation could be seen in a small venule which persisted throughout pregnancy. In the thirty-third week the venules showed very marked distention (A/V 1:10) with many sacculations and a perivenular hazy exudate (figure 9). At this time the conjunctiva showed diffuse "hyaline" infiltration. In the thirty-fourth week patient went into spontaneous labor and delivered a 2,300 gm. stillborn monster with encephalomeningocele. Re-examination one month after the pregnancy showed disappearance of the arteriolar constriction and venular distention. The tissue still contained "hyaline" but no edema.

5. *Intravascular Erythrocyte Aggregation.* In the first trimester no erythrocyte aggregation was usually present in the nondiabetic pregnant woman. In the second trimester red cell aggregates could be seen in the smaller venules and occasionally in the capillaries. In the latter part of the third trimester the erythrocyte aggregation could be observed in the arterioles in approximately 50 per cent of the cases, making the blood flow intermittent in the arterioles as well as in the larger venules. Occasionally static blood cells could be seen in some smaller venules. The red cell aggregates occasionally occluded the narrow ends of terminal arterioles for a second but rarely for longer periods of time. Simultaneously a progressive decrease in the rate of cell flow in arterioles, capillaries and venules (table 3) took place during pregnancy. By contrast, various degrees of erythrocyte aggregation were observed in the diabetic patients during the first trimester. It was common finding that this erythrocyte aggregation was marked by transient arteriolar occlusion at the first visit to the clinic. After two to three weeks the degree of erythrocyte aggregation decreased coincidently with the establishment of better control of diabetes. During the third trimester most (83 per cent) diabetics showed severe arteriolar aggregation with significant decrease in the rate of cell flow (Grade 3) (table 3). In the remaining 17 per cent of the cases, aggregates were observed in the capillaries and venules (Grade 2). In the diabetic cases, showing marked venular distention, the blood flow might stagnate with the settling of aggregates in the venous part of the capillaries and the smaller venules. Such thrombi were observed in eleven diabetic cases during the third trimester. They occasionally persisted for weeks in the same vessel segment. In two cases the configuration of that part of the venular wall, situated close to the occlu-

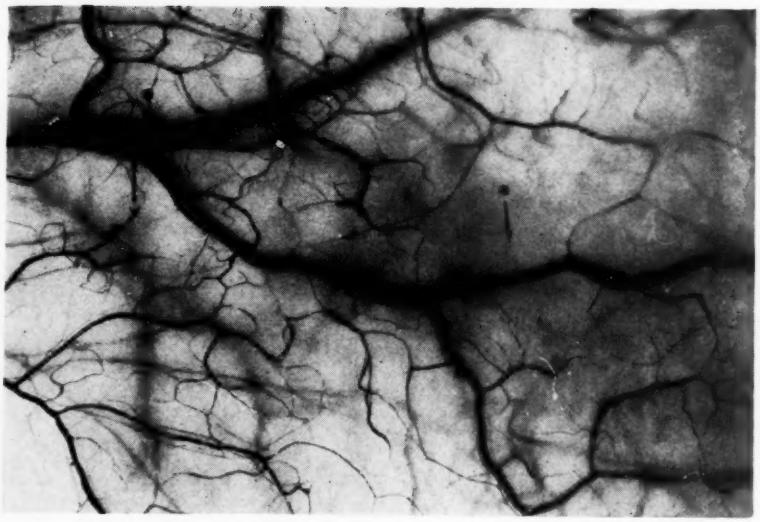

FIG. 9. Case B. J. Diabetic pregnancy during third trimester. Note the markedly changed A/V ratio (1/2) and the perivenular exudation (e).

TABLE 3

Linear velocity (mm./sec.) of red blood cells during early third trimester

	Pregnant diabetics*	Pregnant nondiabetics*	Nonpregnant females*
Terminal arterioles	0.035	0.062	0.10
Capillaries	0.011	0.032	0.052
Collecting venules (30 micra)	0.017	0.045	0.063

*Average of ten cases

sion, showed progressive changes during the period of study. No over-all association was found between the degree of aggregation and the venular distention or the duration of the pregnancy.

6. *Protein Changes.* The protein changes taking place during normal pregnancy are exemplified in figure 10. The changes were significant during the third trimester. The alterations were characterized by a lowering of the total protein, a progressive decrease of albumin and possibly a slight increase in the α_2-globulin. The most consistent change in the globulins appeared to be an increase in the β-lipoproteins during the last weeks of pregnancy. The total amount of glycoproteins and the α_2-glycoprotein fraction did not invariably increase. The same pattern has been seen in five other normal pregnancies. The alterations returned to the prepregnant level approximately four weeks after delivery.

The protein changes occurring during diabetic pregnancy are shown in figures 11 and 12. Besides the same changes, occurring during normal pregnancy, it was repeatedly found that major fluctuations in the serum protein pattern occurred even early in pregnancy. The fluctuations took place particularly in the lipoproteins. The β-lipoproteins appeared particularly labile and increases were great whenever occurring. These changes could often be related to periods of poor control of diabetes. When β-lipoproteins increased markedly, the α-lipoproteins increased simultaneously to a lesser degree. The total glycoproteins likewise showed fluctuations during pregnancy with no relationship to the timing of delivery. When the total glycoprotein increased, the major gain happened in the α_2- fraction. After delivery the different protein fractions tended to return to their prepregnant levels but not necessarily to normal values.

Case Report. A. B., No. 32,789. Diabetes mellitus of eight years' duration without retinopathy. This twenty-six-year-old primipara was first seen at the clinic in the eighth week of pregnancy. She had normal blood pressure. From the twenty-first week patient showed continuously Grade 1 arteriolar constriction. From the twenty-third week arteriolar aggregation was severe, many microthromboses and widespread venous stasis were repeatedly observed from the twenty-seventh week to delivery. Grade 2 conjunctival edema was noted in the twenty-seventh week. Macroscopic edema of the legs was present from the thirtieth week and hydramnios was prominent at the thirty-sixth week. The diabetes was difficult to regulate and patient showed traces of acetone in the urine in the twenty-seventh week along with a blood sugar of 268 mg. per cent. In the thirty-third week the blood sugar amounted to 400 mg. per cent, after which the insulin dose was raised. The episodes in which the diabetes went out of control were associated with simultaneous rises in the β-lipoprotein (figure 11), arteriolar constriction, arteriolar aggregation of erythrocytes, venular thromboses and perivascular exudation. In the thirty-seventh week a 3,800 gm. normal female infant was delivered by cesarean section.

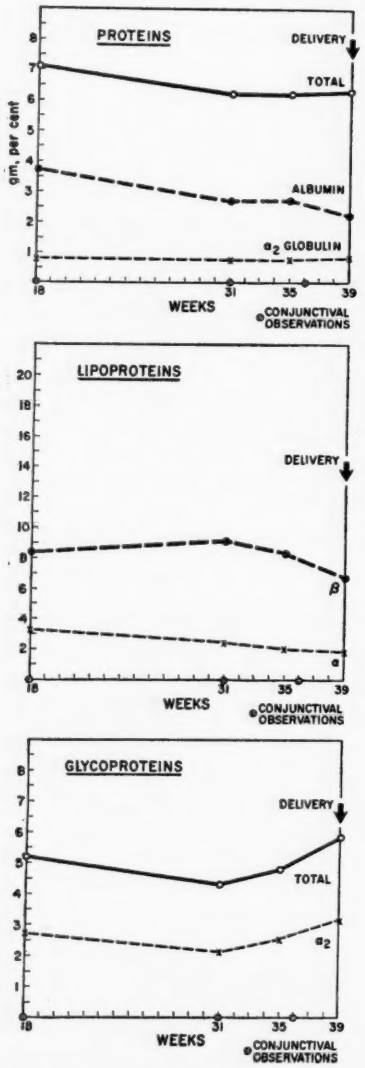

FIG. 10. Case P. J. Protein changes during normal pregnancy.

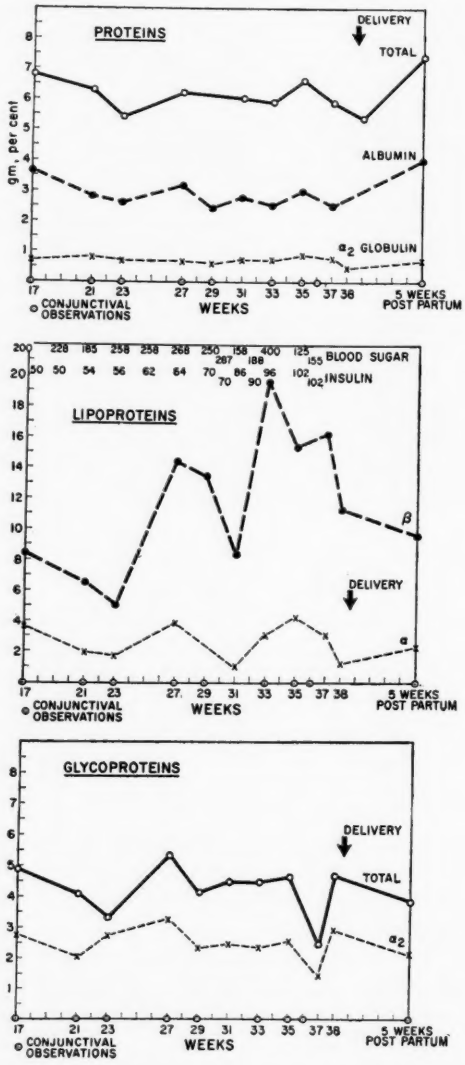

FIG. 11. Case A. B. Protein changes during diabetic pregnancy.

Case Report. E. C., No. 15,957. Diabetes mellitus of nineteen years' duration with moderate diabetic retinopathy, no albuminuria. This twenty-seven-year-old primipara showed albuminuria in the twenty-third and twenty-seventh week, traces of edema and slight hydramnios in the twenty-sixth week. Heart sounds could not be heard in the thirty-third week. The retinal vessels then showed Grade 1 arteriolar spasm. The conjunctival arterioles showed Grade 1 spasm since the eighteenth week, Grade 2 spasm in the twenty-second and twenty-sixth weeks and Grade 3 spasm in the twenty-fifth and thirty-first weeks, with marked arteriolar aggregation. In the eighteenth week the conjunctival venules were distended (Grade 2) and conjunctival microscopic edema was present. The protein, lipoprotein and glycoprotein changes are shown in figure 12. Blood pressure and blood nonprotein nitrogen were normal. The diabetes was well controlled. In the thirty-fifth week a 2,200 gm. macerated dead infant was delivered. Postpartum the arteriolar spasm, the edema and the venular distention decreased, and the aggregation phenomenon and protein distribution improved.

Vascular Pattern Changes in Response to Diabetes. The vascular changes in the pregnant diabetics during the first two trimesters of pregnancy appeared not to differ qualitatively from the changes observed in the

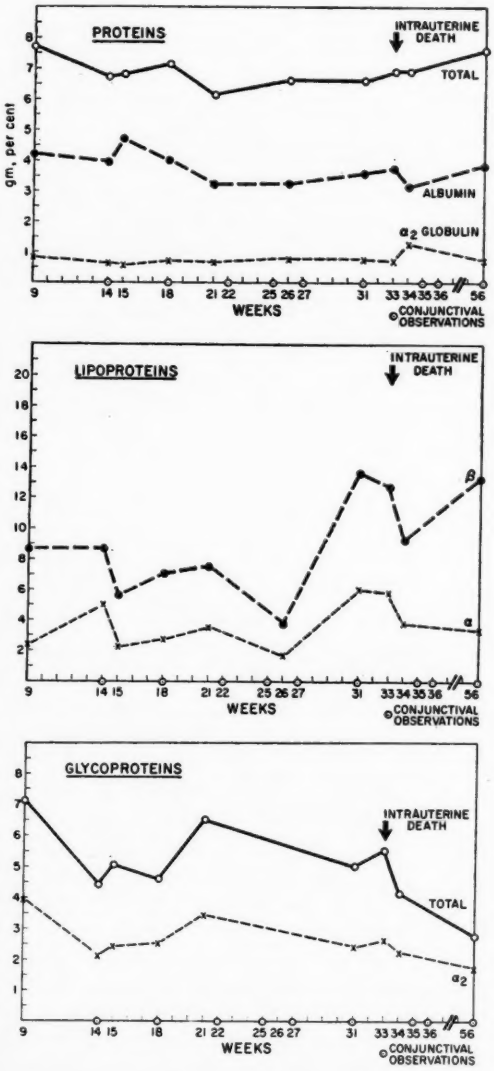

FIG. 12. Case E. C. Protein changes during diabetic pregnancy.

nonpregnant diabetics. However, they were more pronounced quantitatively. The changes occurred in an orderly sequence of events and formed a pattern, designated Vascular Pattern—Change I and II, shown in figures 13a-e. Figure 13a indicates the normal, healthy, functioning vascular bed characterized by a ratio between the diameter of the arterioles and the accompanying larger venules of 1:3-1:2, an optimal velocity of blood cell flow without red cell aggregation, and absence of edema in the perivascular tissue. Figures 13b-d indicate various degrees of Vascular Pattern—Change I, characterized by a slight arteriolar constriction, various degrees of venular distention, exudation through the smaller venules, aggregation of red cells and slowing of linear rate of blood cell velocity. Figure 13e indicates the final stage, designated Vascular Pattern—Change II, characterized by marked arteriolar constriction, closure of most true capillaries, venular attenuation, tissue ischemia, tissue edema and severe arteriolar aggregation of erythrocytes. In this series of thirty diabetic pregnancies, four cases only appeared to present constantly normal vascular pattern. Twenty-one cases showed intermittent or constant Vascular Pattern—Change I, and five cases intermittent Vascular Pattern—Change II. It may be of interest that none of the four diabetics showing constantly normal conjunctival vascular patterns had retinopathy and that two had had diabetes for nineteen and twenty-eight years respectively.

Vascular Changes in Response to Pregnancy. During the latter part of pregnancy the vascular bed of both nondiabetics and diabetics was modified by the following responses: arteriolar constriction, tissue ischemia, venular attenuation or distention, tissue edema, aggregation of red blood cells and slowing of blood flow. During late normal pregnancy the vascular responses passed from those seen in figure 13a to those in 13b or 13e without the intervening stages as indicated by figures 13c and 13d.

The Influence of Complications During Pregnancy Upon the Vascular Bed and the Serum Proteins. Table 4 indicates the complications occurring during pregnancy. In the nondiabetic pregnancy cases, the complicating factors were negligible and consisted only of benign infections in the upper respiratory tract. Among the diabetic pregnancy cases, fourteen had complications. Three showed mild toxemia during the later weeks of pregnancy, three had sterile abcesses from hormone injections, one had a fracture of the lower leg and an abcess, and seven had urinary tract infections. During these periods of illness as well as during periods in which diabetes was difficult to control, it was often but not constantly observed that both vascular, intravascular and protein responses became accentuated. It was not possible to observe any difference between the vascular pattern changes occurring during infections and fracture, and those following poor control of diabetes. In the case of the protein changes during these episodes there appeared to be a difference in the immediate response. When diabetes went out of control the lipoproteins immediately increased, being followed later on by an increase in the glycoproteins. The reverse was the im-

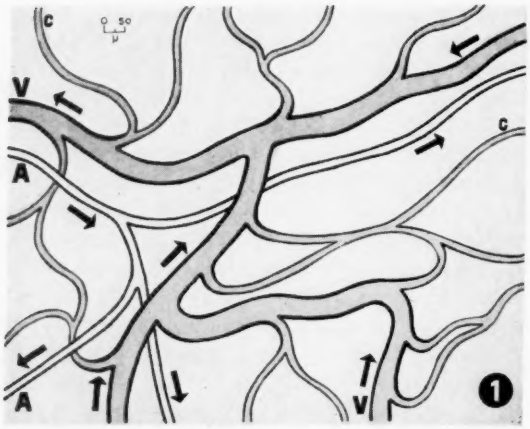

FIG. 13a. Normal vascular pattern.

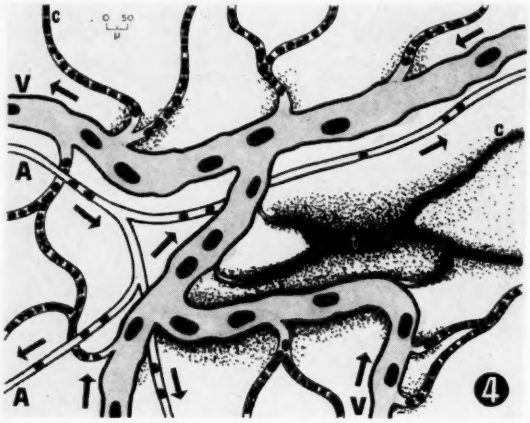

FIG. 13d. Vascular Pattern—Change I, Grade 3.

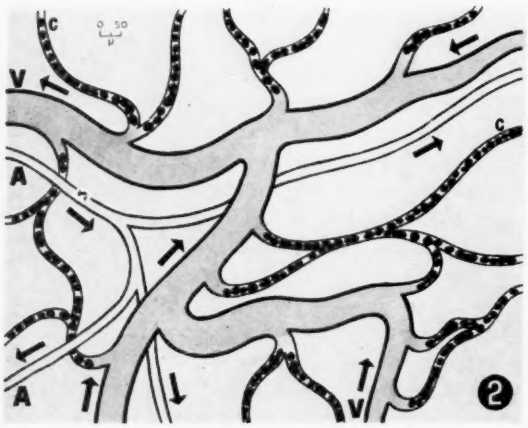

FIG. 13b. Vascular Pattern—Change I, Grade 1.

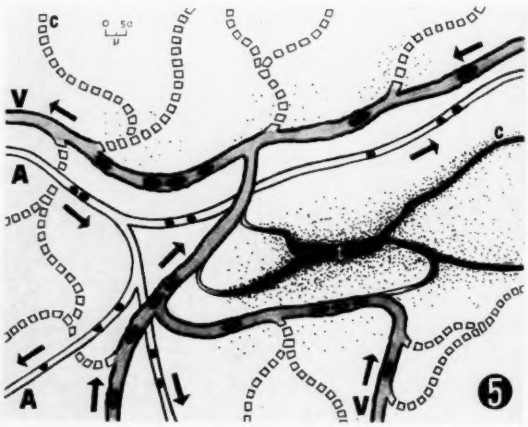

FIG. 13e. Vascular Pattern—Change II.

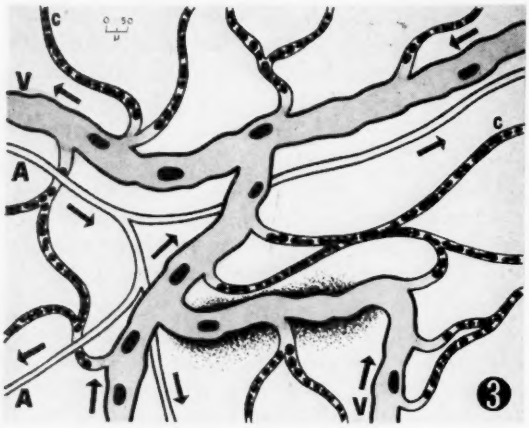

FIG. 13c. Vascular Pattern—Change I, Grade 2.

mediate response to an infection or tissue destruction, presenting a rise of the α_2- and total glycoproteins which a little later was followed by a rise in the lipoproteins.

Relationship Between the Outcome of Pregnancy and Vascular Response Changes. Table 5 surveys the outcome of pregnancies. No fetal loss occurred among the nondiabetic pregnancy cases. Among the diabetic cases were five intra-uterine deaths, one neonatal death, and two with a congenital defect (congenital heart disease, congenital hip dislocation). The relationship between the outcome of pregnancy and the vascular pattern during early part of the third trimester can be seen in table 6. The instances of intra-uterine death occurred only in such diabetic cases showing abnormal vascular responses during pregnancy and particularly frequently in those showing the severe response, Vascular Pattern—

TABLE 4

Complications during pregnancy

	Total number	Number of patients with complications
Nondiabetic pregnancies	30	0
Diabetic pregnancies	30	14*

*Type of complication: toxemia (3), abscess (4), fracture (1), infection (7).

TABLE 5

Outcome of pregnancies

	Number	Living outcome	Intra-uterine deaths	Neonatal deaths	Congenital deformities (in live births)
Nondiabetic	30	30	0	0	0
Diabetic	30	24	5	1	2

TABLE 6

Relationship between outcome of pregnancy and the vascular pattern response

	Number	Intra-uterine deaths
Normal vascular response	4	0
Vascular Pattern—Change I	21	3
Intermittent Vascular Pattern—Change II	5	2

Change II. Since, however, the vast majority of cases showed reversible response changes (87 per cent) this relationship is of little clinical significance. No absolute correlation could be found between any *single* feature of the conjunctival vascular changes and the outcome of pregnancy. The single sign which seemed to be most indicative of prognostic value was arteriolar constriction during the second trimester. Among twelve cases showing intermittent or persistent arteriolar constriction (Grade 1-2) during second trimester, three patients gave birth to stillborn babies.

DISCUSSION

Pregnancy in the nondiabetic woman gives rise to numerous endocrine and metabolic changes. An increased activity of the pituitary, thyroid and adrenal cortex is present. The electrolyte spectrum, the acid-base metabolism and the glucose tolerance may be altered. During pregnancy in the woman with diabetes some of these changes are markedly exaggerated, since the metabolism is grossly altered already at the start. Great variations in control of diabetes are certain during pregnancy and satisfactory adjustment is often difficult.

The changes in the smaller conjunctival vessels and in the serum proteins of pregnant diabetics and nondiabetics can be considered as caused by the altered hormonal equilibrium. In diabetics this hormonal imbalance leads to intermittent periods of abnormal vasomotor changes the damaging effect of which is a marked slowing of linear velocity of blood cells and leakage of plasma fluid through distended venules. Since anatomically and embryologically the conjunctival connective tissue is representative of the subcutaneous tissue, it is likely that similar responses occur elsewhere. The particular tendency of pregnant diabetics to develop clinical edema and hydramnios, and to give birth to edematous infants, may be partly explained by this same mechanism operating in other parts of the body. The finding of some relationship between the degree of vascular responses early in pregnancy and the prognosis of the outcome may also support the thought that similar changes are taking place in the uterus.

The serial microscopic observations of the peripheral vascular bed of pregnant diabetics are particularly interesting in relation to the development of vascular disease in diabetes. Some evidence from this study supports the concept that prolonged periods of abnormal vasomotor responses form a mechanism leading to vascular degeneration: 1) The development of irreversible tortuous elongation of capillaries and configurative venular changes were observed in some cases showing pronounced reversible responses. 2) A progressive change in the perivascular tissue from edema to "hyaline" infiltration was seen. 3) Periods of exaggerated Vascular Pattern—Change I with exudation were often associated with increases in the β-lipoprotein and α_2-glycoprotein fractions of the serum. Even though no rapid progression of retinopathy was noted in this series, it is not unlikely that similar vascular changes might have been observed in the retina if a similar method and magnification could have been employed on this tissue.

SUMMARY

1. The responses of the smaller blood vessels and the serum proteins during pregnancy in diabetic women differ qualitatively and quantitatively from those occurring during pregnancy in nondiabetic individuals.

2. The vascular and serum protein changes in pregnant diabetics (during the first two trimesters) do not differ qualitatively from those in young nonpregnant diabetics.

3. Late in pregnancy in diabetics, changes due to pregnancy per se are superimposed upon changes seen in diabetes.

4. Arteriolar constriction, venular distention, intra-arteriolar erythrocyte aggregation, decreased arteriolar velocity of cell flow and exudation are more severe during pregnancy in diabetic than in nondiabetic women. Each individual diabetic patient varies in her quantitative response.

5. The serum protein changes in diabetics consist, besides those occurring in normal pregnancy, of fluctuations in β-lipoproteins and α_2-glycoprotein fractions.

6. Poor control of diabetes and complicating infections usually lead to accentuation of both vascular, intravascular and protein changes.

7. No absolute correlation was found between the outcome of pregnancy and any single change in the vascular bed or in the protein response during pregnancy in diabetics.

SUMMARIO IN INTERLINGUA

Le Responsas del Vasos Sanguinee Minor e del Proteinas Seral in Diabeticas Pregnante

1. Le responsas del vasos sanguinee minor e del proteinas seral durante le pregnantia differe qualitative-e quantitativemente inter diabeticas e nondiabeticas.

2. Le alterationes vascular e del proteinas seral in diabeticas pregnante non differe qualitativemente durante le prime duo trimestres ab le correspondente alterationes in juvene diabeticas nonpregnante.

3. Verso le fin del pregnantia de diabeticas, alterationes affectuate per le pregnantia per se es superimponite al alterationes associate con diabete.

4. Constriction arteriolar, distention venular, aggregation erythrocytic intra-arteriolar, reduction del velocitate arteriolar del fluxo cellular, e reduction del exsudation — omne iste factores es plus sever in gravidas diabetic que in gravidas nondiabetic. Omne diabetica individual varia in su responsas quantitative.

5. Le alterationes del proteinas seral in diabeticas pregnante consiste, a parte le alterationes que occurre in normal pregnantias, de fluctuationes del lipoproteinas beta e del fractiones glycoproteinic alpha-2.

6. Regulation inadequate de diabete e infectiones complicatori resulta usualmente in un accentuation del alterationes vascular, intravascular, e proteinic.

7. Nulle correlation absolute esseva trovate inter le resultato del pregnantia e le un o le altere specific evento in le vasculatura o in le proteinas seral durante le pregnantia de patientes diabetic.

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Amino Acid Levels and Nitrogen Metabolism

It has been known for a long time that the administration of insulin decreased the level of blood amino acids. The decrease of blood amino acids also occurred in eviscerated animals given insulin and was not related to the blood sugar level nor affected by adrenal demedullation, as had been reported earlier. The fall in plasma amino acid levels was associated with a fall in total free amino acid levels in liver, kidney, and skeletal muscle. As a result of the fall in both plasma and tissue free amino acid levels, the ratio of the tissue-to-plasma levels remained constant. Five amino acids which have been measured individually decreased in about the same proportion in livers and in muscles of diabetic animals. The incorporation of glycine-1-C^{14} into liver glutathione and protein was found to be depressed in the diabetic rat, and was restored to normal in liver slices and diaphragms by the in vitro addition of insulin and glucose. These changes in amino acid levels and metabolism are consistent with the repeatedly expressed view that insulin favors the synthesis of proteins. It has also been demonstrated that there was a decreased incorporation of amino acids into protein and an increased breakdown of proteins and amino acids in alloxan diabetic animals. Adrenalectomy reversed these changes in protein metabolism, along with its well-known ameliorating effect on the diabetes.

From "Enzymatic and Metabolic Adaptations in Animals" by W. E. Knox, V. H. Auerbach, and E. C. C. Lin, in *Physiol. Rev.* *36*:164-254, 1956.

Studies of Abnormal Carbohydrate Metabolism in Pregnancy

The Significance of Impaired Glucose Tolerance

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During the past fifteen years a number of studies have indicated that problems of fetal abnormality exist not only among diabetic women but also among those whom we have come to describe as "prediabetic"; that is, those in whom a diagnosis of diabetes is established at some time later in life after certain experiences in pregnancy.

Allen, in 1939, noted a high rate of fetal loss among prediabetic women.[1] Several authors have subsequently confirmed these observations reporting fetal and neonatal mortality rates ranging from 20 to 39 per cent in offspring of prediabetic women.[2, 3] These rates are higher during the five years immediately preceding the diagnosis of diabetes than during more remote periods. Both fetal and neonatal death rates, however, are appreciably increased among offspring of prediabetic women even thirty years before recognition of diabetes.

An increased frequency of large babies (approximately 10 lb. or more) among prediabetic women has been found in several studies.[2, 3, 4] While the expected general incidence of large babies is about 2 per cent,[5] among prediabetic mothers it is about 20 per cent.[3] In the general population the mortality rate of large babies is about twice that of normal-size babies (i.e., those weighing 5 lb. 9 oz. to 9 lb. 15 oz.[5] The increased incidence of large babies among offspring of prediabetic mothers is most pronounced during the twenty years preceding the diagnosis of diabetes, but it is clearly demonstrable many years earlier.[4]

In addition to increased fetal and neonatal death rates and increased frequency of large babies a likelihood of the following complications of pregnancy is claimed or inferred by some authorities[2, 3, 6] as greater among both diabetic and prediabetic women: premature delivery, polyhydramnios, breech presentation, and toxemia. Increased incidence of congenital defects has been reported by some authors,[2, 6] but has not been found in the experience of others.[3, 7]

The physiological mechanisms involved in the disturbances of pregnancy in relationship to diabetes, prediabetes and impaired carbohydrate tolerance are not clearly understood. It has been shown, however, that:

1. Impaired glucose tolerance (as determined by the standard glucose tolerance test) and glycosuria are more frequent in pregnant than in nonpregnant women.

2. Hyperplasia of the islands of Langerhans occurs in some fetuses of diabetic mothers.

3. Many diabetic women who have complications of pregnancy are prone during the twenty-fourth to thirty-sixth week of gestation to have an increase in serum gonadotropin and a decrease in urinary estrogen and pregnandiol,[8] as compared with the endocrine changes reported in normal pregnancy.[9]

4. In diabetic women the insulin requirement may double or treble during pregnancy.

5. Without insulin treatment, pregnancy is a serious threat to the diabetic woman.

6. Without insulin treatment, a diabetic woman is not likely to produce a viable infant.

7. Some of the disturbances of pregnancy that are often observed in diabetes also occur with increased frequency among prediabetics.

Hoet says that pregnancy exerts a diabetogenic influence.[10] Some of the observations listed above are consistent with this hypothesis. In further support of his theory Hoet quotes a study of the frequency of a family history of diabetes among diabetic women: A family history of diabetes was more frequent among diabetic women who had no or relatively few pregnancies. Conversely diabetic women who had experienced many pregnancies had a family history of diabetes less frequently. Presumably the physiologic derangements

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associated with pregnancy played an important part in the diabetogenesis among the latter women.

Some authorities[1, 10] have suggested that glycosuria during pregnancy should not be regarded as a benign condition. It has been considered as an indication for a glucose tolerance test, and it has been emphasized that performance of these tests should not be postponed until termination of pregnancy. If a glucose tolerance test during the first trimester yields normal results, recommendations have been made that it be repeated during the second and third trimesters. It is also believed that the following groups are candidates for glucose tolerance tests: relatives of known diabetics; those who have a personal or family history of previous pregnancies producing stillbirths, neonatal deaths, or large babies; and those who themselves were large babies.

Hoet recommends maximum tolerated doses of insulin and termination of pregnancy (presumably by cesarean section) at the thirty-fifth week, if there is evidence of impaired glucose tolerance. In his opinion these procedures are necessary to protect pancreatic function of the mother and to prevent hyperplasia of the fetal islands of Langerhans. He does not propose routine use of estrogens in these patients. Hoet has stated that he had applied his regimen of insulin treatment and early termination of pregnancy to more than thirty presumably prediabetic women who had a history of one or more previous pregnancies ending in stillbirth or neonatal death. All of these pregnancies were successful. He also suggested that, in addition to promoting successful outcome of pregnancy, his recommendations may reduce a later development of diabetes in treated women and in their offspring.

If these observations, reported in medical literature, are supported by a carefully controlled study, new vistas may be opened both in reduction of fetal loss and in the prevention of diabetes in mother and child.

METHOD OF STUDY

With the encouragement, advice, and assistance of the Children's Bureau, U. S. Department of Health, Education, and Welfare, and the Massachusetts State Health Department, the Chronic Disease Program of Public Health Service in cooperation with Boston City Hospital and Boston Lying-In Hospital, has undertaken a study to evaluate the effect of insulin treatment on the outcome of pregnancy of women with abnormal carbohydrate tolerance in order to determine if such treatment will:

1. Result in a lower rate of fetal wastage and other complications of pregnancy.

2. Delay the onset of diabetes in pregnant women in the prediabetic stage.

3. Decrease the chance of diabetes occurring in the live births.

At the same time the study is also to determine if women with abnormal carbohydrate tolerance during pregnancy actually develop frank diabetes in subsequent years at a higher rate than women with normal tolerance, and if these women with abnormal tolerance during pregnancy actually have a higher rate of fetal wastage than those with normal tolerance.

It is planned that, should the study confirm the relationships, and should insulin therapy reduce fetal loss and/or prevent or delay development of diabetes, the study methods will point out practical means of screening for more definitive study and treatment those women with abnormal carbohydrate metabolism.

For purposes of this study carbohydrate metabolism is regarded as "abnormal," if any two or more blood sugar readings in a standard 100 gm. three-hour glucose tolerance test equal or exceed the following values expressed per 100 ml. of blood: 110 mg. fasting, 170 mg. at one hour, 120 mg. at two hours, and 110 mg. at three hours. Determinations are made by the Somogyi-Nelson method on venous blood.

These criteria for interpretation of an abnormal glucose tolerance test during pregnancy were established in consultation with a committee of specialists in metabolic diseases.

Except for previously known diabetics, all pregnant women admitted to the prenatal clinics of Boston City and Boston Lying-In Hospitals receive a screening blood sugar test one hour after ingestion of 50 gm. of glucose. A history is taken of diabetes in the patient's family and previous pregnancy data which include history of fetal death, neonatal death, congenital anomalies, prematurity, and toxemia (albuminuria, hypertension, or excessive weight gain), and infants weighing 9 lb. or more at birth.

After these initial observations, women meeting any of the following criteria are selected for a standard 100 gm. three-hour glucose tolerance test:

1. A blood sugar reading of 130 mg. per 100 ml. or more by the Somogyi-Nelson method.

2. History of two or more pregnancies, any two of which resulted in fetal or neonatal death, congenital anomalies, prematurity or toxemia.

3. Having borne an infant weighing nine pounds or more.

4. Having a history of diabetes in the immediate family.

In addition, a random sample of women regardless of screening results are given a glucose tolerance test as

candidates for negative controls. Women meeting the screening criteria as well as the candidates for negative control who have a negative glucose tolerance test in one trimester receive repeat glucose tolerance tests in each subsequent trimester.

All women with an abnormal glucose tolerance test who do not have frank diabetes and who have not reached the thirty-seventh week of pregnancy are selected for the study group. Every second woman in this group is selected for insulin therapy during the remainder of her pregnancy. Thus, four groups of women are under careful observation:

1. Women with abnormal glucose tolerance who received insulin therapy during pregnancy.
2. Women with abnormal glucose tolerance who refused insulin therapy.
3. Women with abnormal glucose tolerance for whom insulin was not prescribed (called positive controls).
4. Women with normal glucose tolerance selected at random as so-called negative controls.

This observation is planned to include prenatal care in the study clinic, postnatal care of mother and child, repeat glucose tolerance tests for the mothers at six to eight weeks post partum, careful examination of mother and child and tolerance testing annually for a period of at least fifteen years, well-baby care for infants and observation with special reference to possible development of diabetes, and autopsy where possible in case of fetal loss.

In a fifth group of overt diabetic women, the course and results of their pregnancy, and the development of their offspring are also under observation.

RESULTS

The studies began in Boston City Hospital on April 5, 1954, and in Boston Lying-In Hospital on Aug. 23, 1954. In the two years, ending in April 1956, 7,316 women have been studied. Of these 2,897 or 39.6 per cent were positive by one or more of the screening criteria. For a part of this period, July 1954 through February 1956, the distribution of positive results by criteria are available and are shown in table 1. The largest group had an abnormal pregnancy history while not meeting any of the other criteria.

About four-fifths of the women who screened positive received at least one glucose tolerance test. Some of them have received repeat tests. Of the 2,256 receiving one or more glucose tolerance tests, 192 have had an abnormal glucose tolerance test according to the study definition. Assuming that those with a positive screening test who were not given a glucose tolerance test would have

TABLE 1

Distribution of women screening positive, by criteria. 5,965 pregnant women (July 1954–February 1956)

Screening criteria	Screening positive	
	Number	Per cent of total screened
Abnormal pregnancy history only	899	15.1
Diabetes in family only	350	5.9
Elevated blood sugar only	314	5.3
Large baby and abnormal pregnancy history	237	4.0
Large baby only	183	3.1
Elevated blood sugar and abnormal pregnancy history	141	2.4
Abnormal pregnancy history and diabetes in family	131	2.2
Elevated blood sugar and diabetes in family	49	0.8
Elevated blood sugar, large baby, and abnormal pregnancy history	46	0.8
All other combinations of criteria	124	2.1
Total all criteria	2,474	41.5

similar tolerance to those tested, it is estimated that the prevalence of abnormal tolerance among all women observed was about 3.4 per cent. Since glucose tolerance tests were given only to those who screened positive, and abnormal glucose tolerance can occur in a woman who was negative, this estimate of the prevalence is a minimum figure. During the coming year it is planned to do glucose tolerance tests on all women, while continuing to collect data according to the original plan, in order to evaluate criteria and determine the error in estimating the prevalence of abnormal carbohydrate tolerance during pregnancy.

In table 2 the results of glucose tolerance tests are shown by the various screening criteria. These also are for the period July 1954—February 1956. As would seem logical, those positive on blood sugar had the highest rate of abnormal tolerance tests. Cases positive on other criteria contribute a smaller but substantial number of abnormal glucose tolerance tests.

Of those with abnormal glucose tolerance, 159 have met the criteria for inclusion in the study. Of these, seventy-nine have been selected for insulin treatment, and eighty have not been selected for treatment, but are being observed as positive controls. In addition seventy-five women with normal tolerance, randomly selected from the prenatal clinic load, are under observation as negative controls.

Only preliminary analysis has been made of the type of abnormality seen in the glucose tolerance test. In table 3 all 192 cases with abnormal tolerance are shown according to the hours at which the blood level

TABLE 2

Results of glucose tolerance tests for women screening positive, by screening criteria (July 1954–February 1956)

Screening criteria	Total glucose tolerance tests performed	Abnormal glucose tolerance tests	
		Number	Per cent of those performed
Elevated blood sugar, large baby and abnormal pregnancy history	36	13	36.1
Elevated blood sugar and diabetes in family	39	8	20.5
Elevated blood sugar and abnormal pregnancy history	106	20	18.9
Elevated blood sugar only	266	42	15.8
Large baby only	141	9	6.4
Diabetes in family only	282	17	6.0
Large baby and abnormal pregnancy history	181	7	3.9
Abnormal pregnancy history only	683	25	3.7
Abnormal pregnancy history and diabetes in family	104	4	3.8
Other combinations of criteria	100	18	18.0
Total all criteria	1,938	163	8.4

TABLE 3

Distribution of women with abnormal glucose tolerance test by study group and hours at which blood sugar level was elevated (April 1954–April 1956)

Hours at which blood sugar level was elevated	Abnormal G.T.T. Study Group							
	Selected for insulin treatment		Insulin not prescribed (positive controls)		Reported after 37th week not classified		Total all groups	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Fasting and second	0	0	0	0	1	3.0	1	0.5
First and second	6	7.6	6	10.0	3	9.1	17	8.9
Second and third	57	72.1	54	67.4	20	60.6	131	68.2
Fasting, second and third	0	0	1	1.3	0	0	1	0.5
First, second and third	15	10.0	15	20.0	7	21.2	38	19.8
At all intervals	1	1.3	1	1.3	2	6.1	4	2.1
Total	79	100.0	80	100.0	33	100.0	192	100.0

reading was elevated. Of the possible combinations 68.2 per cent were elevated only at the second and third hour after administration of glucose. Only six women (3.1 per cent) had elevated blood sugar readings at fasting.

In table 4 the age distribution of study patients is shown. Those with abnormal glucose tolerance appeared to be older than the randomly selected group of women with normal tolerance. More data will be required to determine whether age is really a factor or just an indirect measure of the relationship between glucose tolerance and the number of previous pregnancies. The racial composition of the various groups was very similar.

Through April of this year 138 women with abnormal glucose tolerance tests have delivered in their first observed pregnancy in this study. A number are also in their second observed study-pregnancy. Among those who have delivered, 114 women with abnormal glucose tolerance in pregnancy have had a glucose tolerance test subsequent to delivery. In twelve cases (10.5 per cent) the glucose tolerance was still abnormal according to study criteria at least six to eight weeks after delivery, and several were diagnosed as diabetic. Almost no difference appears between the insulin-treated cases and those from whom insulin was withheld (positive controls), but the numbers of cases are extremely small when the cases are divided into the study groups. No positive results have occurred among the forty women with normal glucose tolerance during pregnancy (negative controls) who have had a post partum glucose tolerance test.

Through April 1956 there have been 192 live births: fifty-two among negative controls, sixty-eight among insulin-treated cases, sixty-eight among insulin-withheld (positive controls) cases, and four among insulin-refused cases. The numbers are as yet too small to draw any conclusions regarding the study objective to determine

whether insulin results in any reduction in fetal wastage. The differences in the average birth weight among infants of the three groups are all significantly different (at the level $P = .99$). The greatest average birth weight is found among infants of women with abnormal glucose tolerance who were not given insulin (positive controls). A brief summary of the significant aspects of the distribution of birth weights by study group is shown in table 5. Three times as many infants weighing nine pounds or more (19 per cent) were born to untreated women with abnormal tolerance as were born to the treated group or to the sample of women with normal tolerance. It is worthy to note that no normal or treated women had babies weighing as much as ten pounds. There also appear to be more small infants among the treated women with abnormal tolerance than among the sample of normals, but the difference is not statistically significant (at the level $P = .99$). It must be reiterated that these data can in no way be considered definitive.

At this stage of the study they are only indicative of what might come out of this study, and they have been presented only as illustrations, since considerable interest has been expressed in the progress of this study.

It is anticipated that screening will be continued for a period of several more years during which patients meeting the criteria will be accepted for study. When sufficient cases have been accumulated, a report on fetal wastage among these women will be presented. It is anticipated that these women and their children will be observed for at least fifteen years to determine the rate at which diabetes develops among women and children in the various study groups.

SUMMARY

1. Abnormal carbohydrate tolerance in pregnant women is the subject of a long-term study undertaken in April 1954. This study is designed to evaluate the effect of insulin treatment of these women during pregnancy by reducing the rate of fetal wastage and other complications of pregnancy and in delaying onset or preventing diabetes from developing in later years in mother and child.

2. Preliminary results are presented. The prevalence of abnormal carbohydrate metabolism, as indicated by interpretation of a glucose tolerance test, is estimated to

TABLE 4

Age distribution of study patients (April 1954–April 1956)

	Abnormal Carbohydrate Tolerance				Normal CHO Tolerance (negative control)	
	Selected for insulin treatment		Insulin not prescribed (positive controls)			
Age	Number	Per cent	Number	Per cent	Number	Per cent
15-19	1	1.3	2	2.5	13	17.3
20-24	13	16.4	12	15.0	26	34.7
25-29	17	21.5	11	13.7	23	30.7
30-34	24	30.4	21	26.3	6	8.0
35-39	12	15.2	15	18.8	6	8.0
40 and over	12	15.2	19	23.7	1	1.3
Total	79	100.0	80	100.0	75	100.0
Average age	31.4		32.8		24.9	

TABLE 5

Birth weights of live births to study patients by study group of mother (April 1954–April 1956)

	Infants of mothers with normal carbohydrate tolerance (negative controls)		Infants of mothers with abnormal carbohydrate tolerance			
			Mother treated with insulin*		Insulin not prescribed (positive controls)	
Birth weight	Number	Per cent	Number	Per cent	Number	Per cent
5 lb. 8 oz. or less	2	3.8	6	8.8	4	5.9
5 lb. 9 oz.–8 lb. 15 oz.	47	90.4	58	85.3	51	75.0
9 lb.–9 lb. 15 oz.	3	5.8	4	5.9	10	14.7
10 lb. or more	0	0	0	0	3	4.4
Total	52	100.0	68	100.0	68	100.0
Average birth weight	6 lb. 14 oz.		7 lb. 2 oz.		7 lb. 11 oz.	

*Excludes infants of four women for whom insulin was prescribed but not given.

be at least 3.4 per cent of the 7,316 women screened in the Boston City and Boston Lying-In Hospitals.

3. Among 114 women with abnormal glucose tolerance in pregnancy who also had a post partum glucose tolerance test, twelve (10.5 per cent) still had abnormal tolerance tests post partum.

4. Three times as many infants weighing nine pounds or more were born to women with abnormal carbohydrate tolerance for whom treatment was withheld (positive controls) as were born to women with abnormal tolerance who were insulin treated, or to a sample of women with normal tolerance (negative controls). These results are prelminary, being based on small numbers of cases.

5. The number of women with abnormal glucose tolerance during pregnancy who have delivered is too small to draw any conclusions regarding fetal wastage at this time.

SUMMARIO IN INTERLINGUA

Studios del Metabolismo Anormal del Hydratos de Carbon Durante le Pregnantia. Le Signification de Imperfecte Tolerantia de Glucosa

1. Anormal tolerantia de hydratos de carbon in gravidas es le thema de un studio a longe vista initiate in april 1954. Iste studio es destinate a evalutar le effecto de cursos de insulina pro gravidas in reducer le grado de guastage fetal e altere complicationes del pregnantia e in retardar o prevenir le declaration de diabete in annos subsequente tanto in le matre como etiam in su prole.

2. Resultatos preliminar es presentate. Le incidentia de anormalitate del metabolismo de hydratos de carbon es estimatemente, secundo un interpretation del test de tolerantia pro glucosa, al minus 3,4 pro cento del 7,316 feminas investigate al Hospital Municipal e al Maternitate a Boston.

3. Inter le 114 feminas con anormal tolerantia pro glucosa durante le pregnantia e in qui tests de tolerantia pro glucosa post parto esseva obtenite, dece-due (10,5 pro cento) continuava monstrar resultatos anormal post parto.

4. Tres vices plus infantes con pesos de nove libras o plus nasceva ab feminas con anormal tolerantias pro hydratos de carbon in qui le tractamento non esseva usate (subjectos de controlo positive) que ab feminas con anormal tolerantias pro hydratos de carbon qui recipeva le tractamento a insulina o que ab un gruppo specimen de feminas con normal tolerantias pro hydratos de carbon (subjectos de controlo negative). Iste resultatos es preliminar, proque illos es basate super un basse numero de casos.

5. Le numero del feminas con anormal tolerantia pro glucosa durante lor pregnantia e qui esseva observate usque al termino non es satis grande pro derivar conclusiones in re le guastage fetal a iste tempore.

ACKNOWLEDGMENTS

Grateful acknowledgment is made of the assistance and valuable contributions of all members of the staff of this project. The guidance and suggestions of the Consultants to our research studies have been of inestimable value. Our consultants are: Dr. Eli Friedman, Dr. Samuel B. Kirkwood, Dr. Arnold Lazarow, Dr. Francis D. W. Lukens, Dr. Alexander Marble, Dr. Duncan Reid, Dr. Gerald R. Rice, Dr. Henry T. Ricketts, Dr. Clement A. Smith, Dr. Richard P. Stetson, Dr. DeWitt Stetten, Jr., Dr. Benjamin Tenny, Jr., and Dr. Priscilla White.

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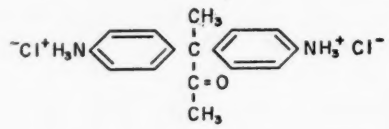
Effects of Amphenone in Patients with Disturbed Carbohydrate Metabolism

Joseph J. Hoet, M.D., Albert E. Renold, M.D., Roy Hertz, M.D., and George W. Thorn, M.D.

In 1952, Hertz and co-workers selected amphenone (3, 3-di(p-aminophenyl) butanone-2 dihydrochloride,* figure 1) as a compound of potential biological interest because of its structural relation to synthetic estrogens.[1]



3,3-di(p-aminophenyl) butanone-2 dihydrochloride
FIGURE 1

The compound exhibited a partly progestational, partly folliculoid activity in experimental animals and, in addition, surprisingly produced enlargement of both the adrenal and the thyroid glands. This adrenal enlargement was due to hypertrophy of the cortex and, when compared with tissue from normal animals, the adrenal cortical cells from amphenone-treated rats were greatly enlarged and appeared loaded with lipid material, particularly in the region of the zonae fasciculata and reticularis. However, in contrast to the hyperplastic appearance of their adrenals, amphenone-treated animals exhibited symptoms indicative of hypoadrenocorticism, thus suggesting that amphenone administration might have resulted in a hyperplastic but hypoactive adrenal cortex. Conclusive evidence of an inhibitory effect of amphenone on corticoid secretion was then obtained in the hypophysectomized dog since the administration of the compound suppressed the appearance of Porter-Silber chromogens in the adrenal vein, despite maximal adrenocorticotrophic hormone stimulation.[2, 3]

The clinical trial of amphenone has so far been limited to (a) the short term (two to four days) use of the compound as a potential means of assessing the role of adrenal cortical secretions in certain disease states and (b) the more prolonged (up to one month) administration of amphenone in an attempt to produce therapeutic suppression of adrenal cortical function in the presence of hyperadrenocorticism or of metastatic mammary carcinoma. The compound has been administered to a total of approximately sixty patients at dose levels of 3 to 10 gm. per day, and the results obtained may be summarized as follows. In patients with adrenal cortical carcinoma, amphenone administration results in a profound suppression of the biosynthesis or release of all three major types of biologically active adrenal cortical steroids: 17-hydroxycorticoids with glucocorticoid activity, aldosterone and 17-ketosteroids.[4, 5, 6, 7, 8] In patients with normal adrenal function, a decreased urinary excretion of aldosterone with concomitant sodium diuresis can be readily shown;[7, 9] a decreased secretion of 17-hydroxycorticoids is clearly apparent only in the presence of exogenous adrenocorticotrophic hormone stimulation;[6] a clear-cut inhibition of 17-ketosteroid secretion has yet to be demonstrated.

In addition to action on the adrenal cortex, amphenone influences the human thyroid in a manner similar to thiouracil[4, 13] and exhibits undesirable side effects, particularly when administered for prolonged periods.[4] When administered for two to four days these side effects have been limited to anorexia, nausea and sedation. More prolonged administration was further complicated by skin rashes, methemoglobinemia and suggestive evidence of hepatotoxicity.[4]

* Amphenone was previously thought to have the following structure: (1, 2-bis(p-aminophenyl) -2-methylpropanone -1-dihydrochloride). When a supply of the compound was made available to us for the continuation of the present studies by the Upjohn Company, Kalamazoo, Michigan, the probability of the structure represented in figure 1 was indicated. This structure has now been established (Korman, J., and Olson, E. C.: *Journal of Organic Chemistry*, in press; Bencze, W., and Allen, M. J.: *Journal of Organic Chemistry*, 22:352, 1957.

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It is the present opinion of the authors that amphenone itself is not a suitable drug for the therapeutic inhibition of glucocorticoid secretion in patients with normal adrenal function. However, evident clinical interest is attached to the possibility of obtaining a reversible inhibition of adrenal cortical secretion in, for instance, patients with diabetes mellitus. Since amphenone derivatives are being synthesized and may prove to be more suitable, it would seem appropriate to record the observations made to date on the effects of amphenone on some manifestations of glucocorticoid activity and particularly in patients with disturbed carbohydrate metabolism. Most observations were incidental to studies in which amphenone was administered for other purposes. Some of these studies have been reported elsewhere.[5, 6]

METHODS

These studies were performed in the metabolic ward of the Peter Bent Brigham Hospital. Amphenone was administered by mouth and generally given in divided doses at intervals of two to four hours throughout the twenty-four hour period. Urinary 17-hydroxycorticoids were determined by the method of Reddy[11] and urinary 17-ketosteroids by a modification of the method of Drekter and co-workers.[12] Plasma 17-hydroxycorticoids were measured by the method of Nelson and Samuels.[13] Urinary glucose levels were measured enzymatically by the method of Froesch and Renold.[5] Since the urinary metabolites of amphenone are colored, are reducing substances and are at least in part lipid-soluble, special precautions must sometimes be taken for chemical determinations during and immediately following periods of amphenone administration. Treatment of the urine with combined cation and anion exchange resins (IR 120 and IR 45 at neutral *p*H) removed the greater portion of these metabolites. This latter procedure was used prior to urinary glucose determinations to avoid excessively high enzyme blanks.

OBSERVATIONS

Effect of amphenone in two patients with adrenal cortical carcinoma. The effect of amphenone on glucose levels in blood and urine has been followed in two patients with metastatic adrenal cortical carcinoma. S. D. (P.B.B.H. 5H689) a forty-one-year-old housewife with florid Cushing's syndrome including diabetes mellitus, hypertension, muscular wasting, striae, osteoporosis, rounding of the face, amenorrhea and loss of scalp hair, was given amphenone on two occasions. The detailed case history has been previously reported.[5] Amphenone administration resulted in a profound decrease in blood and urine 17-hydroxycorticoids and in a remarkable change of the diabetic state as illustrated in figure 2. Amphenone was administered by mouth, an initial dose of 1 gm. being followed by 500 mg. every three hours throughout the first day, and by 500 mg.

ACUTE EFFECT OF AMPHENONE ON URINARY EXCRETION OF 17-HYDROXYCORTICOIDS AND ON INSULIN REQUIREMENT IN A PATIENT WITH ADRENAL CARCINOMA

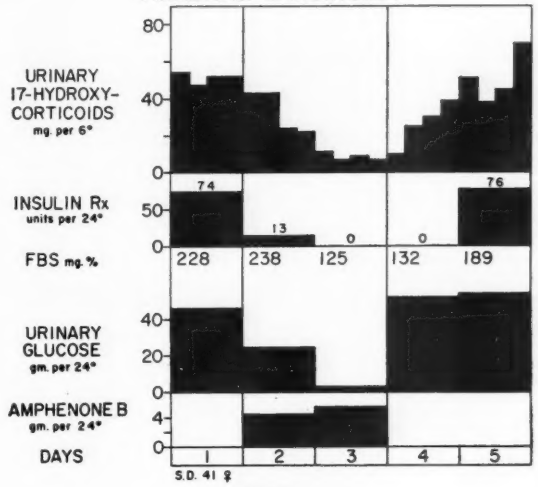

FIGURE 2

every two hours the second day. Marked effects on the urinary excretion of 17-hydroxycorticoids as well as on the measured parameters of carbohydrate metabolism and on the insulin requirements were apparent. Urinary glucose excretion decreased from approximately 40 gm. daily to somewhat over 20 gm. on the first and 2.5 gm. on the second day of amphenone administration, and the fasting blood glucose levels showed a concurrent decrease from values above 200 mg. per cent to 125 mg. per cent. Simultaneously the administered insulin decreased from 74 units on the day before treatment to 13 units on the first and to nothing on the second day of amphenone therapy. After discontinuation of the drug, the pretreatment levels of urinary 17-hydroxycorticoids and urinary glucose were reached within twenty-four hours.

The second patient, S.L. (P.B.B.H. 5J545), a seven-year-old girl, with masculinization of six months' duration, was followed in collaboration with Dr. Sidney Farber and Dr. Edna Sobel from the Cancer Research Institute of the Children's Medical Center in Boston. Exploratory surgery revealed an adrenal cortical carcinoma, and subsequently there appeared pulmonary metastases. After demonstration of some responsiveness of the tumor to adrenocorticotrophic hormone, hypophysectomy was carried out. She subsequently received increasing amounts of amphenone for sixteen days (figure 3). Before amphenone administration she excreted between 70 and 116 mg. of urinary 17-hydroxycorticoids per day. This decreased to 27 mg. per day by the thirteenth day of amphenone therapy, a definite rebound occurring after discontinuation of the drug. Glucose excretion varied from 400 to 600 mg. per twenty-four hours before amphenone therapy (clearly elevated values for enzymatically determined glucose[14]) and continued at that level until the amphenone dose reached 6 gm. daily. From then on, urinary glucose excretion was less than 100 mg. per twenty-four hours until one day after discontinuation of therapy when it returned

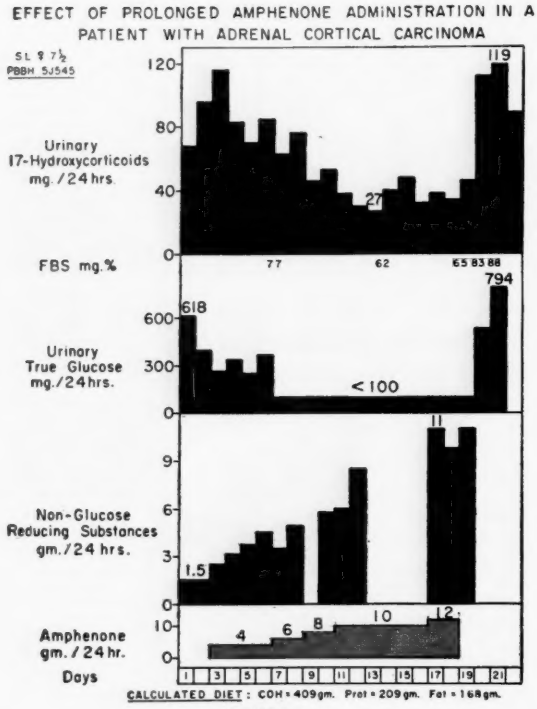
EFFECT OF PROLONGED AMPHENONE ADMINISTRATION IN A PATIENT WITH ADRENAL CORTICAL CARCINOMA
SL ♀ 7½
PBBH 5J545
Urinary 17-Hydroxycorticoids mg./24 hrs.
FBS mg.%
Urinary True Glucose mg./24hrs.
Non-Glucose Reducing Substances gm./24 hrs.
Amphenone gm./24 hr.
Days
CALCULATED DIET: COH = 409gm. Prot = 209gm. Fat = 168gm.

FIGURE 3

to 500 and 800 mg. in twenty-four hours. It should be noted that the patient excreted large amounts of nonglucose reducing substances during the period of amphenone therapy thus examplifying the need for specific glucose determinations during amphenone administration.

Effect of amphenone on the adrenal response to adrenocorticotrophic hormone. K. H. (P.B.B.H. 1J613) a twenty-year-old male with mild Cushing's syndrome due to bilateral adrenal cortical hyperplasia received standardized intravenous infusions of adrenocorticotrophic hormone both before and during amphenone administration. As shown in figure 4, the base line excretion of 17-hydroxycorticoids was 9 mg. per twenty-four hours. Following adrenocorticotrophic hormone administration, the urinary excretion of 17-hydroxycorticoids increased to 47 and 54 mg. per twenty-four hours, a "hyperactive" response compatible with adrenal cortical hyperplasia.[15] Urinary glucose excretion was moderately and significantly elevated at rest and adrenocorticotrophic hormone increased to 9 and 13 gm. per twenty-four hours. When these measurements were repeated before and during adrenocorticotrophic hormone stimulation while the patient was also receiving 6 gm. of amphenone daily, no decrease in the base line excretion of either glucose or 17-hydroxycorticoid was noted. During maximal adrenocorticotrophic hormone stimulation, however, both the urinary excretion of 17-hydroxycorticoids and the urinary glucose excretion increased only to approximately half the levels reached on adrenocorticotrophic hormone alone. Since the dosage of adrenocorticotrophic hormone infused (25 units over eight hours) has been shown to produce maximal stimula-

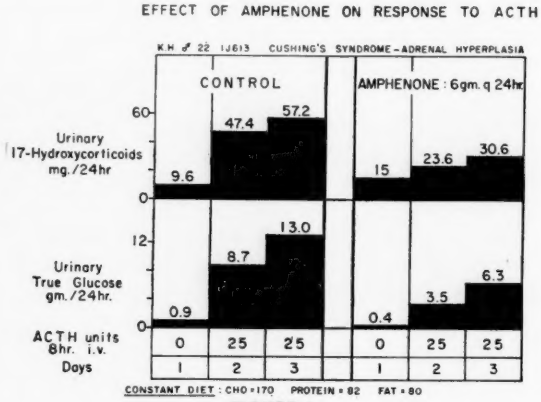
EFFECT OF AMPHENONE ON RESPONSE TO ACTH
K.H. ♂ 22 1J613 CUSHING'S SYNDROME – ADRENAL HYPERPLASIA
CONTROL
AMPHENONE: 6gm. q 24hr
Urinary 17-Hydroxycorticoids mg./24hr
Urinary True Glucose gm./24hr.
ACTH units 8hr. i.v.
Days
CONSTANT DIET: CHO = 170 PROTEIN = 92 FAT = 80

FIGURE 4

tion[16, 17] one may conclude that, in this patient and at this dose level, amphenone administration resulted in an approximate 50 per cent inhibition of glucocorticoid secretion and in an approximate 50 per cent inhibition of its biological effect.

Similar observations were made in subject R. A. (P.B.B.H. 7B948) a forty-year-old male volunteer with normal adrenal function undergoing prolonged stimulation with Armour ACTH gel, 20 units being administered daily by intramuscular injection (figure 5). During the period of adrenocorticotrophic hormone administration a marked gradual increase in the urinary excretion of 17-hydroxycorticoids occurred as well as a definite increase in urinary glucose excretion. The urinary 17-hydroxycorticoid excretion leveled off at 40 to 50 mg. per twenty-four hours and urinary glucose excretion at about 600 mg. per twenty-four hours. When 6 gm. of amphenone was administered in divided doses for one twenty-four-hour period, the urinary excretion of 17-hydroxycorticoids dropped to 20 mg. per twenty-four hours while the glucose excretion reached a minimum of 145 mg. on the following day. After discontinuation of amphenone a "rebound" excretion of 17-hydroxycorticoids occurred with a maximum of 67 mg. per twenty-four hours; this was paralleled by an increase of glucose excretion to 3.2 gm. in twenty-four hours. Again the correlation between adrenal cortical activity and urinary glucose excretion is striking.

Effect of amphenone on the glucosuria of patients with dia-

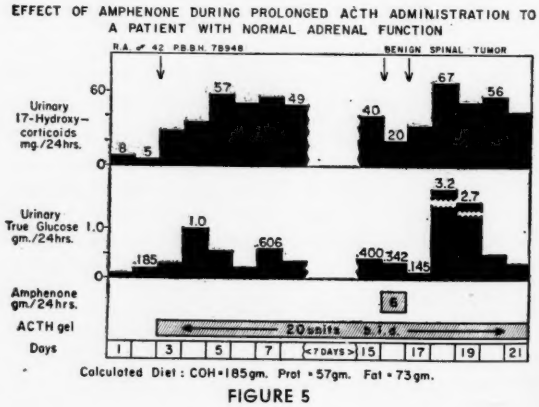
EFFECT OF AMPHENONE DURING PROLONGED ACTH ADMINISTRATION TO A PATIENT WITH NORMAL ADRENAL FUNCTION
R.A. ♂ 42 PB.B.H. 7B948
BENIGN SPINAL TUMOR
Urinary 17-Hydroxy-corticoids mg./24hrs.
Urinary True Glucose gm./24hrs.
Amphenone gm./24hrs.
ACTH gel
Days
20 units
Calculated Diet: COH = 185gm. Prot = 57gm. Fat = 73gm.

FIGURE 5

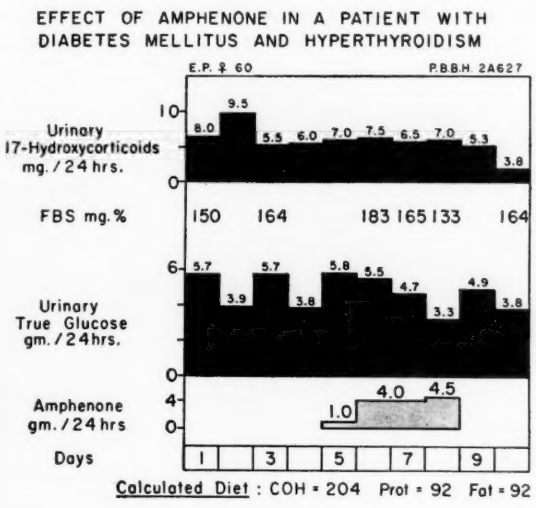

FIGURE 6

betes mellitus. Figure 6 illustrates the effect of amphenone on adrenal cortical secretion and urinary glucose excretion in a sixty-year-old patient with diabetes mellitus and thyrotoxicosis (E. P.; P.B.B.H. 2A627), both of mild degree. Control glucose excretion varied from 3 to 6 gm. daily and the fasting blood glucose varied around 155 mg. per cent. The administration of up to 4.5 gm. of amphenone daily produced little change in the 17-hydroxycorticoid excretion, in the urinary excretion of glucose, or in the blood glucose levels. Also there was no "rebound" after discontinuation of the drug. Similar observations were made in two further patients with diabetes mellitus and in one patient with diabetes mellitus who had previously undergone adrenalectomy and was maintained on a constant dose of cortisone. In no instance did a significant decrease in urinary 17-hydroxycorticoid excretion or in urinary glucose excretion occur. It should be stressed that the total daily amphenone dosage did not exceed 6.0 gm. in any of these patients.

DISCUSSION

Amphenone interferes with the secretion and probably with the biosynthesis[18] of adrenal cortical steroids. It would appear at present that, in man, amphenone administration results in an effective decrease in glucocorticoid secretion whenever endogenous regulation of their secretion is impaired, as in the case for malignant adrenal cortical tissue or during exogenous adrenocorticotrophic hormone administration. The effective, and at times, dramatic suppression of the secretion of 17-hydroxycorticoids under these circumstances is accompanied by equally impressive changes in their biological effects, such as normalization of previously present hyperglycemia and glucosuria, and decreased insulin requirement. The concurrence of decreased levels of 17-hydroxycorticoids in blood and urine with appropriate changes in biological indices of their effectiveness greatly strengthens our confidence in the validity of hormonal measurements.

In the presence of a normal pituitary-adrenal system, however, it is difficult to demonstrate an effective suppression, or indeed any suppression of glucocorticoid secretion as a result of amphenone administration. Again this statement is based both on hormonal measurements and on evaluation of their biological effect. These observations can be reconciled if one considers that, in the presence of a normally functioning pituitary-adrenal system, the adrenal cortical reserve with regard to 17-hydroxycorticoid secretion exceeds basal needs by a factor of the order of magnitude of five to ten.[16, 17] Even a slight initial decrease in circulating 17-hydroxycorticoids, as a result of amphenone administration, would be expected to produce a compensatory increase in adrenocorticotrophic hormone secretion[19] which in turn would lead to a compensatory increase in the secretion of 17-hydroxycorticoids by the adrenal cortex, and in a return to normal of circulating 17-hydroxycorticoid levels. Since, with the doses of amphenone which have been employed, the inhibition of glucocorticoid biosynthesis appears to be of the order of magnitude of 50 per cent (figure 4) a modestly increased adrenocorticotrophic hormone secretion would suffice to restore basal secretion. The finding of adrenal hypertrophy following amphenone administration,[1, 4] and the dependence of this hypertrophy on the presence of the pituitary,[1] support the concept of increased adrenocorticotrophic hormone release during amphenone action.

It is of interest that a suppressing effect of amphenone on the excretion, and presumably on the biosynthesis and secretion of aldosterone, can be readily demonstrated even in the presence of a normal pituitary-adrenal system.[7, 8] This observation may be related to the lesser responsiveness of aldosterone secretion to changes in circulating levels of adrenocorticotropic hormone. The compensatory increase in its secretion postulated above could easily result in normalization of 17-hydroxycorticoid but not of aldosterone levels.

Finally, it should be stressed that as yet the number of patients studied is small and that the side effects of amphenone have been sufficiently bothersome to prevent the use of larger and perhaps adequate doses. Further, more complete evaluation of the use of amphenone as a means of suppressing glucocorticoid production by the adrenal cortex and, therefore, as a means of producing a reversible medical adrenalectomy will have to await the availability of more suitable and less toxic derivatives. Further study of this and related compounds appears clearly indicated.

SUMMARY

The effect of amphenone on glucocorticoid secretion in man, as measured by chemical indices, has been compared with the effect of the compound upon biological indices of glucocorticoid activity. In patients with adrenal cortical carcinoma and in patients undergoing adrenal cortical stimulation with exogenous adrenocorticotrophic hormone, amphenone administration resulted in a striking and immediate suppression of adrenal cortical secretion as measured by both chemical and biological indices. In patients with normal pituitary and adrenal function, however, an effective suppression of glucocorticoid secretion was not achieved. It would appear that the functional reserve of the normal pituitary-adrenal system, with respect to glucocorticoid secretion, is sufficient to overcome the suppression resulting from the doses of amphenone used in this study. Limitation of the dosage was imposed by the occurrence of nausea and sedation. More complete evaluation of amphenone as a means of suppressing glucocorticoid production by the adrenal cortex will have to await the availability of less toxic derivatives.

SUMMARIO IN INTERLINGUA

Effectos de Amphenona in Patientes con Dysfunction del Metabolismo de Hydrato de Carbon

Le effecto de amphenona super le secretion de glucocorticoide in humanos — mesurate per indices chimic — esseva comparate con le effecto del mesme composito super le indices biologic del activitate de glucocorticoide. In patientes con carcinoma adrenocortical e in patientes subjicite a stimulation adrenocortical con corticotropina exogene, le administration de amphenona resultava in un frappante suppression immediate del secretion adrenocortical, mesurate tanto per indices chimic como etiam per indices biologic. In patientes con functionamento normal del glandulas pituitari e adrenal, del altere latere, un efficace suppression del secretion de glucocorticoide non esseva effectuate. Il pare que le reserva functional del normal systema pituitario-adrenal con respecto al secretion de glucocorticoide suffice pro invalidar le suppression que resulta ab le doses de amphenona usate in iste studio. Un limitation del dosage esseva imponite per le occurrentia de nausea e sedation. Un plus complete evalutation de amphenona como medio pro supprimer le production de glucocorticoide per un normal cortice adrenal debe attender usque minus toxic derivatos de illo deveni disponibile.

ACKNOWLEDGMENTS

This research was supported in part by grants from John A. Hartford Foundation, New York City, The National Institutes of Health, United States Public Health Service, Bethesda, Maryland, The Nutrition Foundation, New York City, and Eli Lilly and Company, Indianapolis, Indiana.

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Interaction Between Insulin and Zinc in the Islets of Langerhans

Helmut Maske, M.D., Munich, Germany

In 1943 Okamoto[30, 31] found with the histochemical method which he had developed, that the islets of Langerhans contain much zinc in many animals. He stained sections of fixed pancreas with diphenylthiocarbacide. Following this, Okamoto and Kadota[17, 32, 33, 34] tried to produce experimental diabetes with zinc-binding substances and succeeded with diphenylthiacarbazone (dithizone), 8-oxychinoline (oxin) and a few other related compounds. Wolff, Maske, Stampfl and Baumgarten confirmed Okamoto's findings in producing dithizone-diabetes in rabbits.[40] They were also able to demonstrate that after intravenous injection dithizone is concentrated within the islets of Langerhans as zinc-dithizonate. Stained intravitally in the same manner, frozen sections of pancreas show many fine red granules within the islet cells. In the acinar tissue no zinc-dithizonate is microscopically to be detected.[40]

The intravital zinc stain with dithizone. Further studies have shown that the intravital stain with dithizone is superior to the diphenylthiocarbacide-method on fixed material. The latter method has been described initially by Okamoto for histochemical zinc-detection. With dithizone the original structure is maintained, whereas by the use of Okamoto's method the islets will appear diffusely red and no details can be recognized. In addition, much zinc will be extracted during the fixation procedure of Okamoto whereas by using the intravital stain the results become similar to those obtained with the method of Mager, McNary and Lionetti.[19, 20] These authors have stained frozen post-mortem sections with a solution of dithizone and complex-forming buffer in order to mask other heavy metals which might be present in the cells.

The dithizone solutions for injections have been prepared in the following manner: 100 mg. dithizone (Merck, Darmstadt) were dissolved in a mixture of 1 cc. absolute ethanol and four drops of concentrated ammoniac. This solution was diluted with five to ten parts of distilled water. One hundred milligrams dithizone/kg. body weight were slowly injected intravenously. Five to ten minutes later the animals were sacrificed by intravenous injection of air. Frozen sections of 10-20 μ thickness were made with a freezing microtome, fixed by osmiumtetraoxyde vapors and embedded in glycerol. Counter staining of nuclei may be performed after fixation with hemalaun (figure 1).

Dithizone not only combines with zinc but also forms colored complex-compounds with different bivalent cations. Therefore it became necessary to prove that the color found in the islets of Langerhans after the injection of dithizone is actually zinc-dithizonate. Microspectrophotometric measurements of sections, spectrophotometric measurements and emissions-spectrographic investigations on carbon tetrachloride extracts of the intravitally stained pancreas have proved that the red substance is zinc-dithizonate (figure 2). Lead, cadmium, cobalt and nickel, which also form colored dithizonates at a physiological *p*H-range, have been found to occur in the pancreas in a ratio of approximately one to one hundred with respect to zinc.[15]

The distribution of zinc in the islets of Langerhans. Like Okamoto, McNary and others we have found zinc regularly within the islets of rabbits, dogs, rats, mice, cats and various fishes. In human pancreas the presence of zinc can be demonstrated by post-mortem methods. The only exception among the laboratory animals studied is the guinea pig. In numerous series I was unable to demonstrate the existence of zinc in the islets of Langerhans of guinea pigs. This fact is in line with the results of Marks and Young,[22] showing that the guinea pig pancreas contains less insulin than that of other species. Again, confirming other authors,[6,10,16,39] I have been unable to produce a persistent diabetes with either alloxan, dithizone, or cortisone even in partially pancreatectomized guinea pigs.[28]

It seems in this connection that a certain threshold concentration of zinc is necessary for its histochemical detection. It might be suspected that the concentration of zinc in the islets of guinea pigs is smaller, correspond-

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II. Medizinische Klinik der Universität München, Direktor: Professor Dr. Dr. G. Bodechtel.

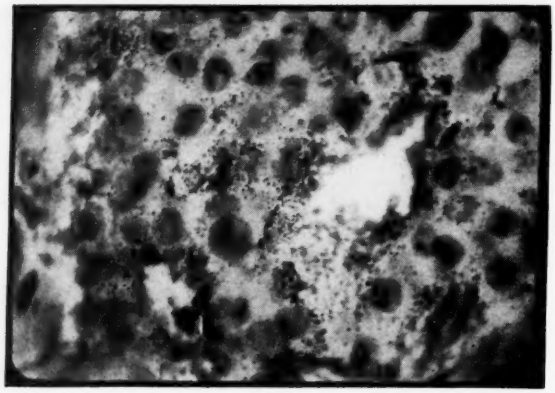

FIG. 1. Islet of Langerhans. The granules correspond to zinc-dithizonate. Injection of dithizone, frozen section, Obj. 25 x, Ok. 10 x.

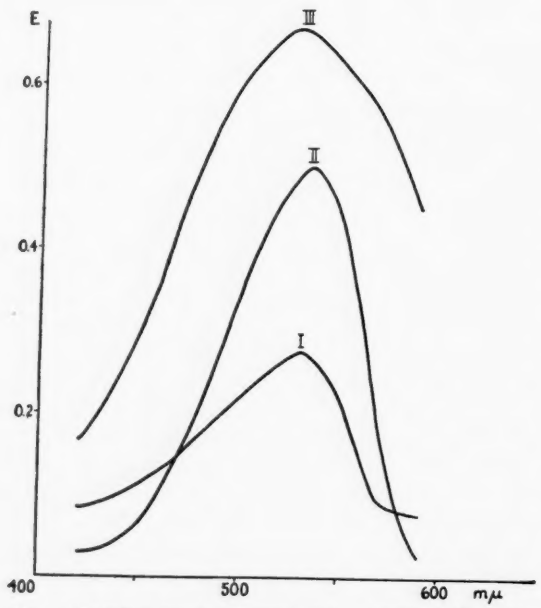

FIG. 2. Extinction-curves measured with a microspectrophotometer, of zinc-dithizonate (I), of carbon-tetrachloride-extracts of pancreas stained intravitally with dithizone (II) and of the granules within the sections (III).

ing to the smaller concentration of insulin. Moreover, the form in which zinc is present seems to play an important role in the identification of this cation. Granules can easily be seen, whereas it is difficult to demonstrate microscopically the same amounts of homogenously distributed zinc. It is therefore possible not to see the zinc in the islets of guinea pigs because of its homogenous distribution there and not in granules as in other species. Another cause for the failure to demonstrate zinc with dithizone may be a stronger binding of the metal to other substances in the body with the result that it is not available for the histochemical reaction. I may conclude thus that the islets of the guinea pig contain not only much less zinc than those of other species, but it may also occur in a different distribution. It cannot yet be stated that no zinc at all is present in the islets.

Of great interest is the distribution of zinc between the different cell types within the islets. In rabbits, dogs and mice the zinc will be found predominantly or even exclusively in the β-cells. In rabbits, made diabetic with alloxan or with dithizone, most of the β-cells are destroyed. In the islets of such animals only small traces of zinc can be found.[23, 40, 41] In the rat β-cells contain small amounts of zinc though α-cells contain much more. Runge,[35] through the dithizone method, has demonstrated that in ducks the α-cells contain as much zinc as the β-cells. Other animals have not been investigated in this respect.

What is the function of zinc in the islets? The importance of zinc for production of experimental diabetes will not be discussed, since Okamoto and Kadota[18, 34] have recently treated this problem. We will confine ourselves to the statement that the presence of zinc in the islets of Langerhans is necessary for the very first phase of diabetogenesis with zinc-combining substances. In this phase cytotoxic substances are concentrated within the cells. Only through this intracellular concentration the diabetogenic substances can exert their cytotoxic action. Our experimental data appear to indicate that the diabetogenic action of alloxan is also mediated by zinc.[23, 24, 25]

Histochemical investigations on the behavior of zinc in β-cells have been performed in rabbits since in this species the zinc is almost entirely contained in the β-cells.

The amount of histochemically demonstrated zinc content within the β-cells varies under different conditions. After administration of glucose or adrenalin considerably less zinc is found than normally.[24, 25, 26] Prolonged glucose loading appears to eliminate most of the zinc from the islets. For example, significantly less zinc could be demonstrated histochemically in most of the islets of rabbits, having received 20 cc. 20 per cent glucose through the stomach tube and 20 cc. 30 per cent glucose intravenously every two hours over a twelve-hour period, than is present normally. Similar results could be obtained three to four hours after the administration of 250 μg. of adrenalin/kg. Under these conditions the intracellular distribution of zinc granules under-

goes changes also. The granules, normally showing an approximately homogenous distribution, are located mainly near the capillaries under conditions which appear to be related to insulin secretion. Twenty-four to forty-eight hours after fasting more zinc is demonstrable than in the fed controls. It should be pointed out that changes in zinc demonstrated histochemically may be simulated by differences in the physical state of the zinc.

These changes of dithizone stained zinc, containing granules, are similar to such which can be observed by employing conventional stains in β-cell-granules under various physiological conditions. Best and Haist[4] and others[2, 3, 5, 42] have correlated these histological changes with the insulin content of the β-cells. It should be noted, however, that the granules demonstrated by dithizone are not identical with those demonstrated by common histological methods. The dithizone granules appear to be greater and more distinct and not as numerous. I have observed also that the histochemically demonstrable zinc content of the islets decreases after application of glucose even before changes in the number of granules by histological stains with the usual methods can be recognized. After more intense glucose loading zinc had disappeared from the islets while granules still could be stained.[26] McNary[19] found no zinc but still granules twelve hours after alloxan administration to rabbits. Therefore zinc represents a more sensitive indicator for functional alterations of the islets than the granules demonstrated with usual histological methods. From all these observations it can be assumed that zinc and insulin in the islet cells may biochemically be related. I have tried to find experimental evidence for this concept.

The intracellular distribution of insulin and zinc. It is of special interest to find out which constituent of the islet cells contains the zinc and whether zinc and insulin are related to each other in some way. Quantitative studies of this problem can only be performed on relatively pure islet tissue. This can be found in certain bony fishes which have isolated giant islets with little excretory tissue, the so-called Brockmann bodies. It is well known from previous studies that these bodies contain a large amount of zinc.[38] The fish islets therefore appeared especially suited for this problem. Together with Munk, Homan, Bouman and Matthijsen we have analyzed a great number of these giant islets of flounders (Pleuronestes). The results of these experiments are published in detail.[29]

In two experiments, 200 and 500 Brockmann bodies of living fishes respectively, weighing a total of 1 gm. and 3 gm., respectively, were freshly homogenized with a Potter-Elvehjem-Homogenizer in isotonic sucrose solution (*p*H 7. 5) and centrifuged at different speeds. All manipulations were done at 0-5° C. Each individual fraction of cell nuclei, mitochondria and cell plasma was analysed. Zinc and nitrogen were estimated quantitatively in each fraction insulin, and in the second experiment also the enzyme succinodehydrogenase (SDH). The different fractions were sedimented twenty to thirty minutes in an MSE-centrifuge at 0° C. with 8,000-19,000 rpm after separating and washing the nuclei at 2,000 rpm (700 x g). Insulin was tested in cross tests with rabbits after rough estimation in mice. Zinc was colorimetrically determined with dithizone[9] and nitrogen was estimated by the micro-Kjeldahl-method. Estimation of SDH was performed in the following way: In the presence of an excess of substrate (succinate) time needed for the discoloration of a colored hydrogen acceptor (2, 6-dichlorphenol-indophenol) was used as a standard for enzyme activity.

In the first experiment the estimation of insulin could not be performed satisfactorily in every fraction. The total amount of insulin and of zinc was found to be greater than in the second experiment. This difference was attributed to the different nutritional state of both groups. The fishes of the first experiment were captured in December, while the second experiment was carried out during the last days of April.

The results of the second experiment are summarized in table 1, and demonstrate the greatest concentrations of insulin and zinc to be present in fractions, presumed to contain mitochondria or microsomes. After washing these fractions, relatively small amounts of insulin and zinc have been found in the supernatant fluid. This fact indicates a close binding of both zinc and insulin to the granules. Further studies of the different fractions showed remarkable differences in their composition. The most striking fact was the identical distribution of insulin and succinodehydrogenase. The greatest amounts of either substance have been found in a fraction centrifuged at 10,000 rpm (9,000 x g), presumably and predominantly mitochondria on account of their succinodehydrogenase content. Sixty-three per cent ± 15 per cent of all insulin has been found in this fraction (figure 3). The concentration of insulin was 4.7 per cent, calculated on its nitrogen content, and should be higher even, considering that the homogenate contained some extrainsular tissue. The zinc content of this fraction was 4 μg./mg. nitrogen ± 22 per cent. The concentration of zinc relative to that of insulin within the system

TABLE 1

The analytical results of preparative centrifugation of fish islets (Maske, Munk, Homan, Bouman, Matthijsen)[29]

	Nitrogen mg.	Zinc microgm.	Insulin units	Succino-dehydrogenase arbitr. units x 10^3	
fr. 1: Sediment after 10 min. centrifugation with 2,000 rpm (700 x g) and washing	13.0 ± 1%	16 ± 33%	3 ± 55%	0.5 ± 23%	Nuclei and unhomogenized tissue
fr. 2: Sediment after 30 min. centrifugation with 10,000 rpm (9,000 x g) and washing	6.2 ± 2%	24 ± 22%	47 ± 11%	3 ± 24%	Mitochondria
fr. 3: Sediment after 30 min. centrifugation with 19,000 rpm (33,000 x g) and washing	4.6 ± 3%	24 ± 31%	8 ± 28%	0.7 ± 25%	Mitochondria and microsomes
fr. 4: Supernatant fluid after the last centrifugation	27 ± 3%	27 ± 44%	9 ± 42%	0	Plasma and microsomes
fr. 5: Washing fluid of fraction 2	2.6 ± 5%	7 ± 65%	6 ± 39%	not estimated	
fr. 6: Washing fluid of fraction 3	3.8 ± 4%	9 ± 65%	2 ± 93%	not estimated	

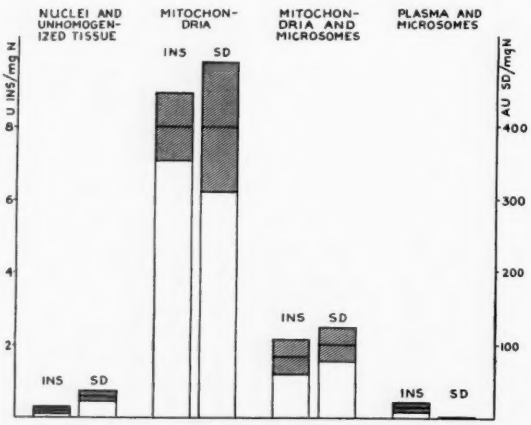

FIG. 3. The concentrations of insulin and succinodehydrogenase in different fractions after preparative centrifugation of fish islets. Shaded areas mean p=0.05.

precipitate plus washing fluid (fraction 2 + 5) has been determined as 1.5 per cent. This is sufficient to precipitate pure insulin at physiological *p*H in the absence of other complex-forming substances.[14] But, in relation to my findings, it appears more probable that zinc is involved in fixing insulin to proteins of granular cores. The question still remains if these granules are uniform insulin-containing mitochondria, as the equal distribution of insulin and SDH appears to demonstrate, or if there are different kinds of granules: mitochondria *and* other insulin-containing granules, which coincidently have the same relation of size and specific gravity.

The fraction centrifuged at 19,000 rpm (33,000 xg) appears to contain a mixture of more microsomes and fewer mitochondria. It holds a smaller amount of insulin and a still higher concentration of zinc than the above discussed fraction. A surplus of zinc is present in respect to the zinc-binding capacity of insulin (8 per cent).

The supernatant which contains plasma and microsomes gives no further evidence since it contains only very small amounts of insulin. The significance of the large amounts of zinc in microsomes and cell plasma containing fractions not histochemically demonstrable with dithizone remains to be clarified.

Comparing the results on fishes with the histological findings in mammalians, especially in rabbits, the fraction containing most insulin and relatively much zinc has to be related to granules which can be demonstrated histochemically with dithizone in respect to size and zinc content.

The failure in histochemical demonstration of smaller particles with even higher zinc content can be explained by a different chemical binding of the metal or by optical phenomena. It is much more difficult to demonstrate with the microscope particles smaller than the wave length of visible light.

The difference to or the identity with granules demonstrated with other histological methods remains un-

clarified. The procedure of commonly used histological methods destroys parts of the cell structure, e.g. mitochondria. Also most of the insulin and part of the zinc are extracted.[1, 19] Knowledge is scanty about the chemical nature of these secretory granules. They appear to consist of protein, but their staining characteristics, e.g., with chromhaematoxylin[11] and with aldehyde-fuchsin,[13] are not related to insulin.[1, 13] Thus the observed and well established relations between these granules and insulin cannot be explained yet. Differences in the behavior of dithizone stained granules in frozen sections and of those stained with the common histological methods under different conditions in respect to the storage of insulin may be explained in two ways: 1. The histochemically demonstrable zinc belongs to cell particles, no longer demonstrable after normal fixation as are mitochondria. 2. Zinc attached to protein cores together with insulin may disappear while the granules persist. Histochemically demonstrated granules may appear larger since they contain insulin and zinc. The above cited calculation of only 4.7 per cent insulin in the fraction with the main insulin content agrees with both considerations. One may observe thus granules with common staining methods, when zinc is no longer demonstrable and when insulin is no longer present in sections.

Zinc and insulin secretion. The presence of considerable amounts of zinc in cell particles containing insulin, and the similar behavior of insulin and zinc under different physiological and unphysiological conditions suggests that the metal is engaged in the insulin secretion. The close relationship in the islet-cells between insulin and zinc allows a comparison with their mutual relations in vitro. The hormone possesses a rather specific and strong ability to form chelate-complexes with zinc and some other metals.[8, 37] The complex formation between insulin and zinc is dependent upon the *p*H. Above a *p*H of 6.0 insulin takes up increasing amounts of zinc out of a surplus in solution with increasing *p*H. At the *p*H of blood (7.3) more than 2 per cent can be bound. Cohn et al.[7] and Hallas-Møller and co-workers[14] have shown that zinc-binding has a strong influence on the solubility of insulin. Insulin, soluble at *p*H above 6.0, becomes insoluble if an excess of zinc is present, and if no stronger complex-forming substances than insulin are present in the solution.[14] Tanford and Epstein[37] have shown that under such conditions the metal is combined with the imidazol groups of the protein-hormone. The insulin-bound zinc may still bind other basic groups, e.g., amino acids, protamine, etc.[8]

On the basis of the rather specific and intensive ability of insulin to form insoluble complexes with zinc, and as a result of our observations in histochemically demonstrated zinc and in fractionating islet tissues it may be assumed that this hormone is stored by interaction of zinc. The places of insulin storage are granules. Before being delivered by the cell insulin must be released from the granules. How does this happen?

The islets of Langerhans are particularly sensitive to an increased supply of glucose. It is well known through many investigations that the delivery of insulin is immediately dependent on the glucose concentration in the pancreatic arteries. An observation of Stier[36] demonstrates that, a few minutes after injection of glucose, islet cells reduced much more triphenyltetrazolium-chloride to red formazan than before injection. The reduction was also greater than in surrounding excretory tissue.[36] Therefore, glucose appears to stimulate far more the metabolism of the islet cells than that of other cells. The reduction of tetrazolium salts to colored compounds in tissues has been related to dehydrogenases, located in mitochondria.[21] Glucose itself does not release insulin from an insoluble zinc complex in vitro. But various intermediary metabolites are known, forming stronger complexes with zinc than insulin, and which therefore possibly act competitively, if greater amounts originate by an activated metabolism and by a greater supply of glucose than in resting cells.

Investigations of the influence of different zinc-binding metabolites on the solubility of zinc insulin have been performed in vitro at *p*H 8.0 and 37° C.[27] Concentration of the tested substances was between 5×10^{-4} and 5×10^{-3} mol. These in vitro studies have demonstrated that citric acid, oxalacetic acid, glutathion, cystein, histidin, and organic phosphorus compounds can bring insoluble zinc-insulin-complexes into solution by releasing the insulin from complexes of pure zinc-insulin or of protamine zinc insulin. All these substances may be available physiologically in islet cells when the metabolism is stimulated. Therefore the concept of an interaction between metabolites and insulin-zinc-complexes in vivo is supported by the observation of competitive interaction between zinc-binding metabolites and zinc-insulin in vitro.

The delivery of insulin from the β-cells in response to blood sugar elevation may thus be pictured as follows: The stored hormone is firmly bound to proteins of granules. Zinc is essentially involved in this process. When the blood sugar rises, the metabolism within the islet cells will be stimulated more than that in other

tissues as the above cited investigations with triphenyltetrazoliumchloride have shown. As a matter of fact, it appears that the cyclophorase-system within the mitochondria of the β-cells is specially involved in the stimulated metabolic processes. The activation of the cell metabolism causes the delivery of insulin from the granules by changing the zinc bond of the hormone. Complex formation of various metabolites of the citric acid cycle, acting competitively with the zinc bound to insulin, may be one of the mechanisms of this process.

SUMMARY

1. A review of the literature about histochemically demonstrable zinc in the islets of Langerhans is presented. Experimental observations on this subject are reported. Under conditions where insulin secretion is stimulated, only small amounts of zinc can be demonstrated. On the other hand more zinc is found when insulin is being stored. The number of zinc-containing granules changes with different physiological states of the islet cells before the quantity of granules demonstrable with other histological methods does.

2. Preparative centrifugation and analysis of relatively pure islet tissue of fishes have shown the main content of insulin to be in the mitochondrial fraction. The same fraction contained adequate zinc for precipitating that amount of insulin. In the main microsomal fraction even a higher concentration of zinc has been found. The relation of the centrifuged cell particles to histological and histochemical findings is discussed.

3. The in vitro chemical relation between insulin and zinc suggests that the metal is involved in insulin storage in vivo.

4. Histidin, cystein, citric acid, oxalacetic acid and organic phosphorus compounds form stronger complexes with zinc in vitro than does insulin. Because of the availability of these substances in an increased islet-cell metabolism, as demonstrated by triphenyltetrazoliumchloride after glucose loading, a similar process is postulated to take place during the delivery of insulin from granules and the islet-cell. Therefore, an increased metabolism resulting from elevated blood glucose levels could stimulate the insulin secretion.

SUMMARIO IN INTERLINGUA

Interaction Inter Insulina e Zinc in le Insulas de Langerhans

1. Es presentate un revista del litteratura relative a zinc e su demonstrabilitate histochimic in le insulas de Langerhans. Observationes experimental concernente iste thema es reportate. Sub conditiones de stimulation del secretion de insulina, solmente basse quantitates de zinc pote esser demonstrate. Del altere latere, quando le insulina es retenite, plus grande quantitates de zinc es a trovar. Differentias del stato physiologic del cellulas insular se reflecte in alterationes del numero de granulos continente zinc ante que illos effectua alterationes del quantitate de granulos demonstrabile con altere methodos histologic.

2. Centrifugation e analyse de relativemente pur tessuto insular de pisces ha monstrate que le contento de insulina se trova principalmente in le fraction mitochondrial. Iste mesme fraction contineva adequate quantitates de zinc pro precipitar le quantitate de insulina presente. In le major fraction microsomal, un concentration mesmo plus alte de zinc esseva trovate. Es discutite le relation del centrifugate particulas cellular a constatationes histologic e histochimic.

3. Le relation chimic inter insulina e zinc in vitro suggere que le metallo es interessate in le immagasinage de insulina in vivo.

4. Histidina, cysteina, acido citric, acido oxalacetic, e organic compositos de phosphoro entra in vitro in le formation de complexos con zinc es plus forte que le complexos de insulina e zinc. A causa del disponibilitate de iste substantias in le presentia de un augmentate metabolismo del cellulas insular — un facto demonstrabile per medio de triphenyltetrazoliumchlorido post cargation con glucosa — il es postulate que un simile processo occurre durante le liberation de insulina ab granulos e le cellulas insular. Per consequente, un augmento del metabolismo como resultato de elevate nivellos sanguinee de glucosa esserea capace a stimular le secretion de insulina.

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A Study of Serum Lipoprotein and Cholesterol Determinations in 901 Diabetics

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For over 100 years pathologists have recognized that the lipid laden intimal plaque impinging upon the lumen of the arteries is the characteristic feature of atherosclerosis. The dogma that it was an inevitable process of ageing resulted in a paucity of progress in this field. This theory was finally abandoned when pathologists found infants and children with intimal lipid streaks and elderly patients with minimal or no atherosclerosis. In 1913 Anitschkow[1] stimulated a great deal of experimental work with his classic discovery that a lesion simulating atherosclerosis could be produced by feeding cholesterol to rabbits, and thus helped to categorize this disease as one of lipid metabolism.

The lipids of the blood have long been known to exist in combination with other substances. More than a quarter of a century ago Macheboeuf[2] isolated from serum a preparation containing large amounts of cholesterol and phospholipids. Both are water soluble due to their association with protein, and without this association such hydrophobic substances would lose their solubility in plasma. Gofman and his colleagues,[3] from the Donner Laboratory of the University of California, demonstrated in 1950 that low density lipoprotein molecules in plasma could be made to undergo flotation in the ultracentrifuge. This marked the beginning of a renewed interest in research as to the status of serum lipids in relation to atherogenesis.

BACKGROUND OF THE STUDY

On recommendation of the National Heart Council, the United States Public Health Service formed a Committee on Lipoproteins and Atherosclerosis and sponsored a joint laboratory study of the beta-lipoprotein. Dr. John Gofman of the Donner Laboratory, University of California; Dr. Max Lauffer and Dr. Martin Hanig, Department of Biophysics, University of Pittsburgh; Dr. Irvine H. Page, Research Division of the Cleveland Clinic Foundation; Dr. Frederick J. Stare and Dr. George V. Mann, Department of Nutrition, Harvard School of Public Health, were in charge of the four laboratories. Dr. J. Franklin Yeager of the National Heart Institute served as coordinator, and Mr. Felix E. Moore of the Biometrics Research Section, National Heart Institute, as statistical consultant. Lipoprotein determinations of the serum of approximately 15,000 normal subjects were made. The results of this study will be reported in the near future. A one to two year follow-up of these normals, evaluating the development of coronary disease, has already been reported by the joint laboratories.[4]

Premature atherosclerosis is frequently found in several disease entities including diabetes mellitus, hypothyroidism, nephrotic syndrome, biliary obstruction and familial xanthomatosis. Diabetes is now considered as an accelerator but not as the cause of atherosclerosis. Because of this relationship between diabetes and atherosclerosis, the authors studied the serum of a group of almost 1,200 diabetics. A preliminary report[5] of these studies on 618 patients was presented in 1952.† In this study are reported the 901 patients whose serum was analyzed after the four laboratories in the National Project had standardized their procedures. The cases studied prior to the standardization were discarded from the present series. There were almost 300 such cases and many of them were included in the preliminary report. In addition, a follow-up of these diabetic patients has been completed. A paper similar to the one published by the Committee on Lipoproteins and Atherosclerosis, will be forthcoming. It will examine the correlation between ensuing atherosclerotic complications and the original lipid determinations.

PURPOSE OF THE STUDY

The purpose of this study was to determine any correlation between certain characteristics and complica-

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†The present report had to be delayed due to the untimely death of Dr. Joseph Barach in March 1954 and the recall to active naval duty overseas of Dr. Alexander D. Lowy, Jr.

tions found in diabetics and the concentration of various lipoprotein molecules. Simultaneously cholesterol determinations were carried out, and compared to the lipoprotein values. Cholesterol is a part of the lipoprotein molecule and is present in different quantities in each S_f fraction.

SUBJECTS

The 901 patients of this study included clinic patients who had been followed for several years in the diabetic out-patient department of the University of Pittsburgh Medical School, and private patients of the authors and Dr. Louis Pennock of Pittsburgh, Pa. There were 276 private patients including six Negroes, and 624 clinic patients including 204 Negroes and one Chinese. Of these 901 patients 268 were white males, 422 white females, 54 Negro males, 156 Negro females, and one Chinese male. No extensive report will be made on the Negro males and the Chinese patient, since the group is small. Clinic and private patients were combined for the analysis of data because no significant differences in their lipids could be detected.

The age distributions in the four sex-race groups were quite comparable and the median age for the whole group was 52.5 yr. The youngest patient was fourteen years old and the oldest eighty-seven years. Age adjustments in the analysis that follows were not made on account of the similarity of age distributions.

Mann[6] has shown that lipoproteins act similarly to elevated cholesterol during acidosis, and during recovery from it the values return to normal. None of the patients studied was in coma, and very few had acetonuria. It was felt therefore, that the cholesterol and lipoprotein changes in acidosis would not affect the data.

PROCEDURE

A careful history, with emphasis placed upon family history, age of onset of diabetes, type of diet, use of insulin, insulin reactions, diabetic coma and weight change, was obtained from each patient in addition to the history of cerebrovascular accidents, myocardial infarction, angina pectoris or the symptoms of arteriosclerosis obliterans. The patients were then examined carefully. The eyes were examined with particular reference to the fundi and to the presence or absence of arcus senilis. The blood pressure was determined, and heart and abdomen were examined. The lower extremities were surveyed for any evidence of arteriosclerosis obliterans. If the posterior tibial or dorsalis pedis pulsation was decreased, in a warm room, various other tests were employed, or consultation with the Peripheral Vascular Department was obtained. Likewise, patients with questionable fundi were observed in the Ophthalmology Department. Further information was obtained from medical records which were available on most of the subjects.

Thirty cubic centimeters of blood were then collected without regard for the time of the last food ingestion. The serum was sent to the Biophysics Department of the University of Pittsburgh where it was stored at 0-5° C. until the S_f 12-20 and S_f 20-100 determinations could be carried out in the ultracentrifuge.[7]* The serum was then analyzed by the Abell-Kendall-Levy-Brodie[8] method for cholesterol.

On the initial examination a blood sugar, urinalysis, X ray of the chest, lateral X ray of the abdomen and X rays of the lower extremities were performed. The coded results of the history, physical examination, laboratory and X-ray data were then transferred to IBM cards for statistical study.

CONTROL GROUP

To find a control group for comparison with the diabetic patients the authors decided to use that portion of the normal subjects in the National Study whose serum was processed in Pittsburgh, as was the serum of the diabetic patients. Available for the control group were 1,528 men and 151 women.

METHODS OF ANALYSIS

After examination of the relationship of the cholesterol and S_f values to each other and to pertinent characteristics of the diabetic patients it was decided not to use any index to summarize the measures. This decision was based on the low linear relationships, which the measures showed to each other, and on the fact that summarizing them in any additive fashion might obscure relationships between measures and characteristics. Correlation coefficients (r) for each of the measures with the others showed that the highest relationship existed between S_f 12-20 and S_f 20-100 with (r) equal to 0.62. The next highest was cholesterol and S_f 12-20 with (r) equal to 0.51, and lowest was cholesterol and S_f 20-100 with (r) equal to 0.23.

To evaluate the effect of sampling variation on the comparisons made in this paper, a test based on the Student's "t" distribution was used. The null hypothesis tested was: Diabetic patients with a certain characteristic

*A technical discussion of the ultracentrifuge, entitled "Lipoproteins and Ultracentrifuge Techniques" and sponsored by the technical group meeting in Chicago on Nov. 8, 1952, is available from Dr. J. Franklin Yeager, National Heart Institute of Health, Bethesda, Maryland.

showed an elevation of blood lipids not greater than could be expected by chance alone. The test is therefore based only on one half of the "t" distribution. The testing procedure employed was applied to one measure at a time. Where there are similarities in the behavior of the three measures the probability statements do not account for the fact that such consistent outcomes are not likely to occur on the basis of sampling variation. Some of the differences observed may therefore be of more interest than probabilities derived from these tests would indicate. In general this testing procedure is not very helpful in detecting small deviations from the null hypothesis, due to the high variability of the data.

The standard deviations for the three measures can be found in table I.

With groups of this size and standard deviations such as these one cannot expect to detect true differences of less than fifteen consistently, and in some cases differences considerably greater may not be noticed. It remains to be seen whether differences smaller than this amount are of clinical interest.

Only two of the three lipid measures were available for several patients included in the study. This resulted in slight variations in the number of patients with a specific characteristic in the graphs that follow.

To our knowledge, the Negro patients in this study are the only ones on whom lipoprotein determinations are available. The data were analyzed in the preliminary report of 1952 without regard for race, while in this study findings on Negroes are evaluated separately. The results on Negro females were slightly different from the white females and, considering the numbers in these two groups, these differences may be meaningful. The findings on the white patients will be presented separately in the first part of the paper, and the findings on the Negro females will only be summarized.

RESULTS ON WHITE PATIENTS

The six charts in Graph I show the age in five or ten year groups and its effect on cholesterol and S_f values. A number of relationships may be seen. The mean cholesterol values for males and females are approximately the same at all ages covered by our data as those of the corresponding controls. The S_f 12-20 values change somewhat with increase in age. The diabetic males show some reduction during the middle years and the females a sharp rise in the early years with a subsequent leveling off, and a decline in the oldest age group. Part of this decline with age is to some degree also present in the other measures, and may be due to the effect of increasing mortality on the older age groups. For the S_f 12-20, the differences between diabetic males and the normal Pittsburgh controls are not definite, but the findings in females are consistently higher than the controls at all ages. The S_f 20-100 shows considerable variation with age, with a marked decline in later years. The mean values of the diabetic males are lower and the average values of the diabetic females higher than their corresponding controls.

KIMMELSTIEL-WILSON DISEASE

Rifkin[9] has reported that Kimmelstiel-Wilson disease can now be differentiated from other types of kidney disease by finding fat-staining refractory cells in the urine. Staining technics have shown that microscopic lesions of the kidney in intercapillary glomerulosclerosis or Kimmelstiel-Wilson disease are due to lipid deposits. In this analysis patients with this complication had higher mean values for cholesterol and the S_f molecules. Similar findings have already been described by Engelberg, et. al.[10] Graph I shows that with all measures the mean values were higher than the diabetic controls without Kimmelstiel-Wilson or any other diabetic complication.

The elevated lipid values of the cases with Kimmelstiel-Wilson disease would affect the findings of patients with retinopathy, hypertension, and other atherosclerotic complication. It was decided therefore, to remove them when these characteristics were studied.

TABLE 1

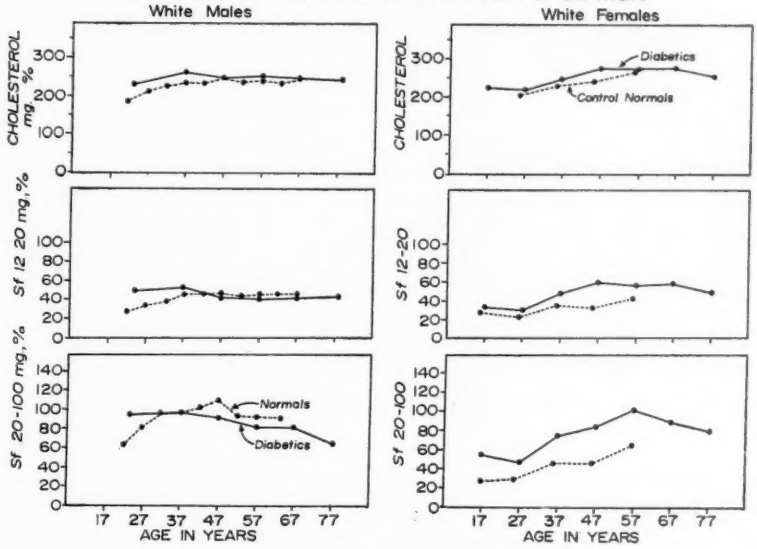
Means and standard deviations of cholesterol, S_f 12-20, and S_f 20-100 by sex and race for diabetics and normals

	Number of patients	Cholesterol		S_f 12-20		S_f 20-100	
Diabetics		Mean (mg.%)	S.D. (mg.%)	Mean (mg.%)	S.D. (mg.%)	Mean (mg.%)	S.D. (mg.%)
White males	268	246.4	57.6	44.4	26.6	85.5	73.8
White females	422	266.3	61.7	55.3	38.5	88.0	67.4
Negro females	156	270.5	67.5	48.1	37.8	62.7	80.9
Pittsburgh Normals							
White males	1,528	233.3	46.3	42.7	23.2	95.7	72.2
White females	151	242.5	*	35.6	*	49.0	*

*Not available.

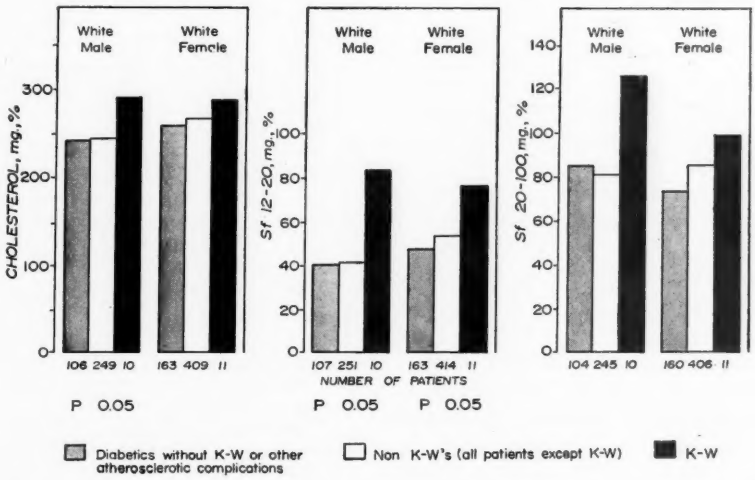
LIPIDS WITH AGE

GRAPH I. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES BY 5 OR 10 YEAR AGE GROUPS IN DIABETIC PATIENTS AS COMPARED TO A NORMAL CONTROL POPULATION



KIMMELSTIEL-WILSON CASES

GRAPH II. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES IN KIMMELSTIEL-WILSON CASES BY SEX, AS COMPARED TO THE NON K-W DIABETIC PATIENT AND A DIABETIC CONTROL GROUP



HYPERTENSION

Hypertension in this study was defined as a systolic pressure over 140 mm. of Hg. and a diastolic pressure over 90 mm. Hg. A systolic under 100 was considered low and the remaining cases were categorized as normal. As expected, hypertension was quite prevalent in the 901 diabetic patients. With the Kimmelstiel-Wilson cases removed, 15 per cent of the remaining

white males and 24 per cent of the remaining white females were hypertensive.

Hypertension appears to have some relationship to the lipid values, and it is a measure of considerable clinical significance in diabetics. For this reason differences in the proportion of patients with hypertension in the various groups were taken into account when analyzing the data. The adjusted mean values showed essentially the same results as the unadjusted data, and therefore the latter are presented.

Graph III shows the cholesterol and the S_f levels of the patients according to the different blood pressure groups. For the females, the mean values of hypertensive diabetics were found to be considerably higher than those of diabetics with normal and low blood pressure. In the males we are dealing with small numbers of patients in the low and high blood pressure groups. The small differences between mean values for the male patients with normal and high blood pressure are difficult to interpret.

RETINOPATHY

The fundi of all the patients were examined following dilation of the pupil with 1 per cent paredrine. The retinopathies were classified as follows:

Grade O: Normal fundus

Grade I: Microaneurysms and/or hemorrhages but so few that they might be missed with cursory examination.

Grade II: Many easily seen microaneurysms and/or hemorrhages.

Grade III: Waxy exudates present with or without aneurysms and/or hemorrhages.

Grade IV: Retinitis proliferans and its complications.

Since Grades I and II, and Grades III and IV showed gradation of the same lesion and had similar lipoprotein values, they were grouped together. This resulted in three final groups of: no lesions, designated Group A; Grade I plus Grade II, designated Group B; and Grade III plus Grade IV, designated Group C.

It can be seen in Graph IV that patients with retinopathy, Group C, showed consistently higher readings in the cholesterol and S_f 12-20 than patients without retinopathy (Group A). For the S_f 20-100 the differences are not meaningful. Group B showed only a slight elevation of the mean values for the S_f molecule when compared to Group A.

ATHEROSCLEROTIC LESIONS

Forty-nine white males and fifty-four white females who had a history of, or findings consistent with, atherosclerotic complications were examined. By definition atherosclerotic complications consisted of a cerebrovascular accident, angina pectoris, myocardial infarction, or arteriosclerosis obliterans. They were found in 13 per cent of the white males and 10 per cent of the white females. In a normal population such complications are approximately seven times more frequent in men than in women. The complications are listed in table 2 in the various combinations in which they occurred.

Due to the small number of cases and different types of lesions, it was considered best not to analyze the data by type of cardiovascular complications. All these conditions, despite affecting various parts of the body, have similar pathology. Warren[11] has emphasized that a parallelism exists between the severity of lesions in the aortic trunk, and those of the coronary and cerebral vessels. At times, however, atheroma may be present in one group of vessels and none in any of the others.

Graph V shows the results in cases with atherosclerotic complications as compared with diabetic patients without any of these complications. The white females show a definite elevation of all measures as compared with diabetics without complications. The male group, however, shows no differences in the mean S_f values and only a slight elevation in the mean cholesterol.

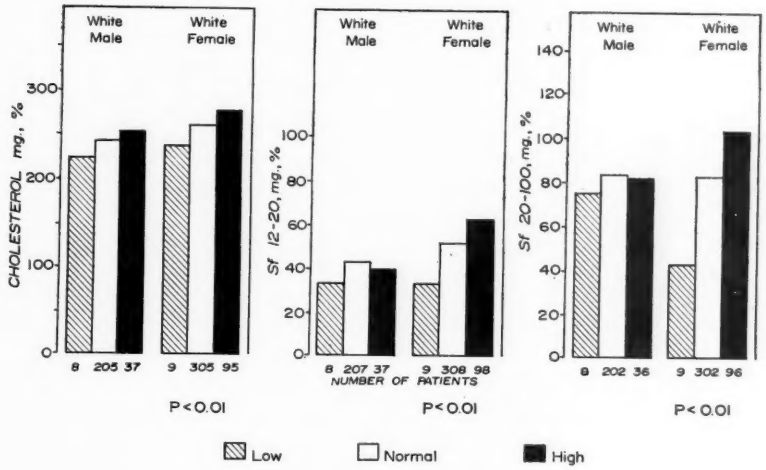
TABLE 2

Number and type of cardiovascular complications in white male and white female

Complication	White male	White female
Cerebrovascular accident	3	4
Myocardial infarction	4	1
Arteriosclerosis obliterans	21	12
Angina pectoris	14	27
Arteriosclerosis obliterans and cerebrovascular accident	1	1
Angina pectoris and myocardial infarction	2	3
Angina pectoris and arteriosclerosis obliterans	2	5
Arteriosclerosis obliterans and myocardial infarction	1	
Angina pectoris and cerebrovascular accident		1
Angina pectoris, myocardial infarction and arteriosclerosis obliterans	1	

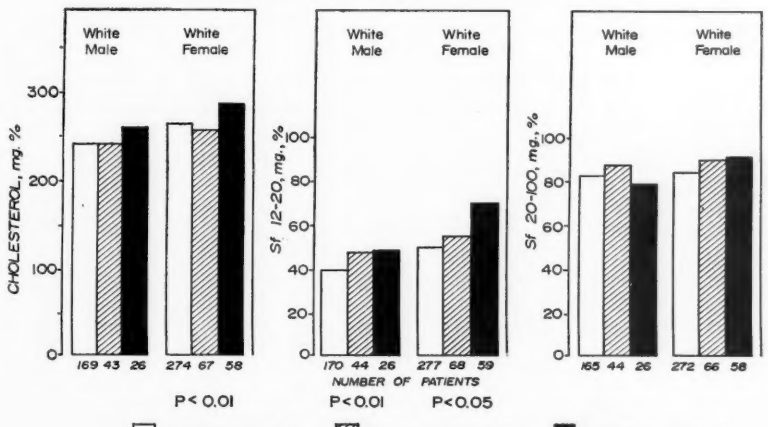
BLOOD PRESSURE

GRAPH III. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES IN DIABETICS BY SEX, COMPARING HIGH, NORMAL AND LOW BLOOD PRESSURE.



DIABETIC RETINOPATHY

GRAPH IV. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES OF DIABETICS WITH DIFFERENT DEGREES OF RETINOPATHY BY SEX.



INSULIN THERAPY

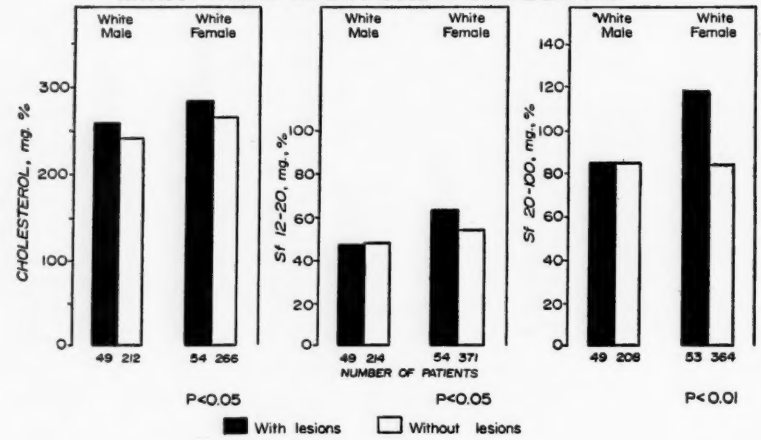
Graph VI compares the lipid measures of patients taking insulin with those not taking insulin. Only a slight elevation of lipids can be seen in the males taking insulin. In general, patients on insulin therapy have a more severe diabetes than those not taking insulin, but some patients would be under better control with small doses of insulin.

ARCUS SENILIS

Arcus senilis is a form of lipid deposition in the peripheral cornea associated with senescence but sometimes occurring early in life. It appears as an opaque grayish white arc, usually beginning in the lower or upper portion of the cornea. This lipid material is not the same as subcutaneous fat, since it does not stain black with osmic acid, but does with Scharlach R and

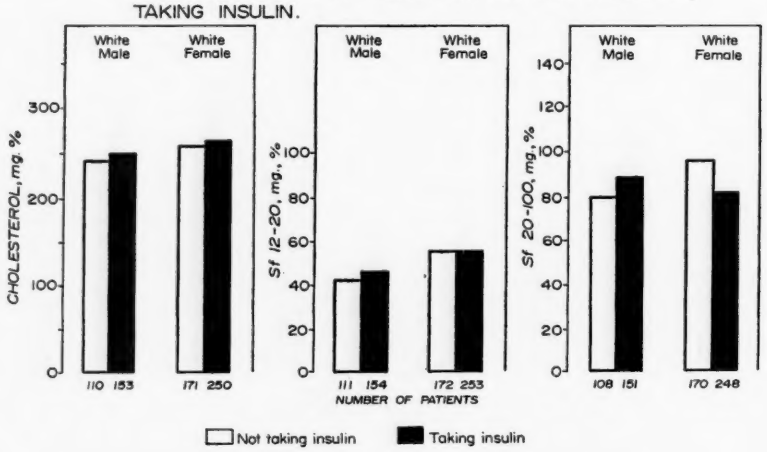
ATHEROSCLEROTIC COMPLICATIONS

GRAPH V. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES BY SEX IN DIABETIC PATIENTS HAVING HAD A MYOCARDIAL INFARCTION, ANGINA PECTORIS, ARTERIOSCLEROSIS OBLITERANS OR A CEREBRAL VASCULAR ACCIDENT AS COMPARED TO THE DIABETIC GROUP WITHOUT THESE CARDIOVASCULAR COMPLICATIONS.



INSULIN THERAPY

GRAPH VI. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES BY SEX IN DIABETIC PATIENTS TAKING INSULIN AND THOSE NOT TAKING INSULIN.



Sudan III, and possibly is a cholesterol ester. Verse[12] has produced experimentally lipid arcs in corneas of rabbits by feeding them cholesterol.

Arcus senilis therefore may be a manifestation of lipid disorder. It was found in 14 per cent of the white males and 12 per cent of the white females. The three lipid measures were not elevated in patients with arcus senilis.

DIABETES CONTROL

Among the 690 patients forty-eight were white males and sixty-five white females with good control and cooperation. Forty-two were white males and seventy-six white females with poor control and cooperation. The remaining 459 white patients were either intermediate, unknown, or were new patients who could

not be properly categorized. It was considered that this characteristic, if it had any effect, could be detected by examining the two extreme groups. Good control implied that the patient had never been in coma except at the time of initial discovery of diabetes, was given insulin when needed, that urines were sugar free, and fasting blood sugars were usually below 150 mg. per cent. Poor control implied that the patient had been in and out of coma, that urine specimens showed two plus to four plus sugar, that he had acetonuria at intervals, and that fasting blood sugars were frequently over 180 mg. per cent.

The results obtained when patients with different control and cooperation were examined are shown in Graph VII. In all instances except for the cholesterol readings in the white females, the patients with poor control and cooperation show higher mean values. The differences in the male group were more pronounced than in the female group.

OBESITY

The height and weight of the patients were determined while they were fully dressed. An estimation of ideal weight was made from the Metropolitan Life Insurance charts[13] according to the patient's build. All patients weighing ten or more pounds below normal were considered as underweight, those within nine pounds of normal weight as normal, and those ten or more pounds over normal weight as obese. Thus there were three groups for comparison. If any correlation between weight and lipid values did occur it should have been possible to detect consistent differences through this classification. The cholesterol values were not elevated in the overweight patients. The S_f 20-100 determinations were elevated for obese patients of both sexes, but in the S_f 12-20 this elevation was found in females only. The trend in these instances was as would be expected if obesity were positively related to the level of lipoprotein in the blood. The comparison of the overweight to the underweight individuals was significant at the 5 per cent level for the S_f 20-100 in males and the 1 per cent level for the S_f 12-20 and S_f 20-100 in females.

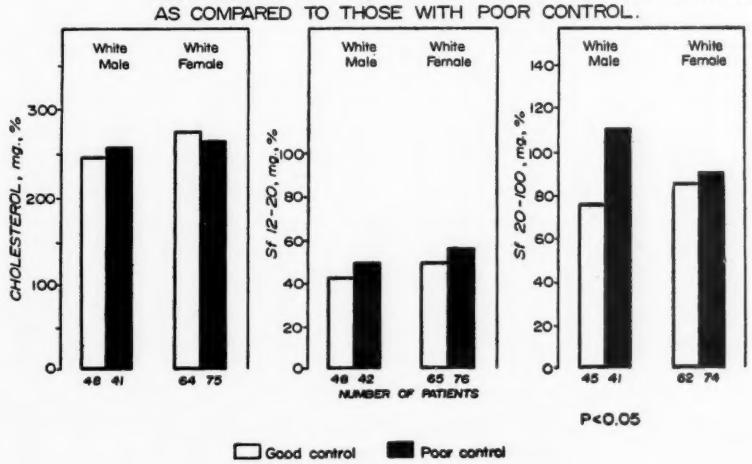
It was of interest that only 40 per cent of the white males who were not hypertensive were obese, while 60 per cent of the males who were hypertensive were obese. The females also showed the trend with 62 per cent not hypertensive being obese, and 80 per cent hypertensive being obese. This relationship of obesity and hypertension has been known for years.

CALCIFICATION OF VESSELS

Until recently the clinician has used the term "arteriosclerosis" to denote hardening of the arteries, and this term referred to both intimal and medial lesions. They have been separated only in recent years as two definite entities which may occur concurrently. The elastic arteries such as the aorta are usually affected by intimal sclerosis, while the arteries of the extremities show also the medial changes or Mönckeberg's sclerosis. Both lesions may appear calcified when seen by X ray, the only differ-

DIABETIC CONTROL

GRAPH VII . MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES BY SEX IN DIABETIC PATIENTS WHO ARE UNDER GOOD CONTROL AS COMPARED TO THOSE WITH POOR CONTROL.



ence being that the medial calcification may give a tubelike appearance, while the intimal changes may be patchy in type. X-ray examination of calcified blood vessels in the body is an indirect method of studying atherosclerosis.

Roentgenograms of the thoracic aorta, abdominal aorta and extremities were carried out and the calcification classified as Grades I, II, III, IV. Barely visible streaks were considered Grade I, a tubular calcified vessel was considered Grade IV and Grades II and III were intermediate. For the purpose of statistical analysis the calcified areas of blood vessels were finally divided into two groups: thoracic plus abdominal which represents atherosclerosis in large vessels, and femoral plus tibial which may represent not only calcified atherosclerosis but also some medial sclerosis. In dividing the patients who showed some calcification in this way we do not account for those who had calcification at all sites. The analysis therefore does not tell us if different sites of calcification have different mean values.

The roentgenograms were read by a qualified roentgenologist and rechecked by one or both of the authors. In the final analysis the data were grouped as to represent no calcification or some calcification. In the males, Graph VIII shows no clear-cut relationship between lipid values and calcification of any of the blood vessels. For the females, however, we can observe a large and consistent rise of blood lipids with the presence of calcification. This occurs regardless of whether the aorta or the leg vessels are involved.

RESULTS ON NEGRO PATIENTS

In evaluating the Negro group in relation to cholesterol and lipoprotein only fifty-four male patients were available and, as mentioned previously, it was considered that with data as variable as these measures, a group of this size is not sufficient for statistical analysis. But 156 females were included in the study and the findings of this group are of interest.

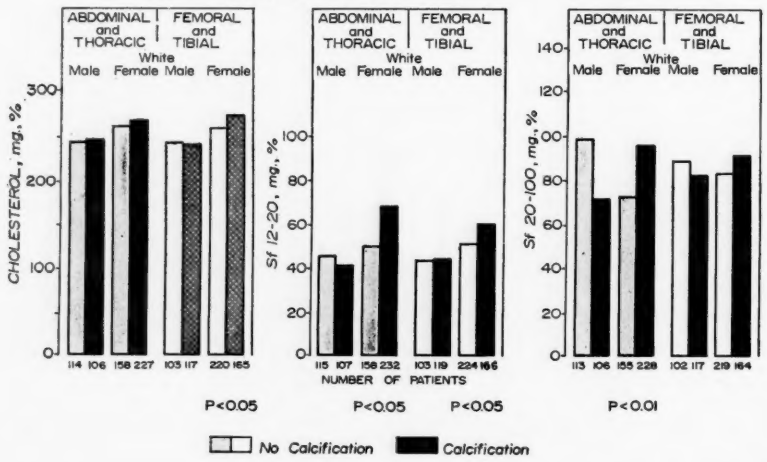
Some similarities and differences were observed between the Negro females and the white females. In both groups there was some elevation of all three lipid measures in patients with retinopathy, atherosclerotic complications and calcification of blood vessels. In addition, Negro females had elevated values for those having poor control and those taking insulin. In patients taking insulin the elevation of the cholesterol and S_f 20-100 values was slight. The Negro females, unlike the white females, did not show an elevation of blood lipids in patients with hypertension.

In both the Negro and white females, the findings on patients with arcus senilis and obesity show nothing conclusive. Only four cases of intercapillary glomerulosclerosis were found among the Negro females, and therefore nothing definite can be said about the effect of this condition on lipid values, although an elevation was observed.

Arcus senilis appeared more frequently in Negroes than in white patients, which confirmed an interesting clinical impression of the authors. The age distribution being about the same, 14 per cent and 12 per cent of the

CALCIFICATION OF BLOOD VESSELS

GRAPH VIII. MEAN CHOLESTEROL, Sf 12-20 AND Sf 20-100 VALUES IN WHITE DIABETIC PATIENTS WITH AND WITHOUT CALCIFICATION OF VARIOUS ARTERIES OF THE BODY.



white males and white females respectively showed arcus, while 75 per cent of the Negro males and 59 per cent of the Negro females showed arcus senilis.

DISCUSSION

It was hoped when this study was initiated, that a correlation between the serum lipoproteins and the development of certain diabetic characteristics and complications might be established. Only in the female diabetics did the findings substantiate the hypothesis of a positive relationship between atherosclerotic complications and elevated lipid values. The diabetic females showed consistently higher mean values when compared to normal females in all age groups. They also showed elevations in the average values of the blood lipids with the presence of Kimmelstiel-Wilson disease, diabetic retinopathy, cardiovascular complications, hypertension, obesity, and vessel calcification. Some of these elevations were statistically significant. The males, except in the Kimmelstiel-Wilson cases and the diabetic control comparison, showed no meaningful results. In addition, the males did not show elevation of the mean lipid values when compared to the normal male population. The Negro females, when compared to the white females, showed similar but less definite results, and this may be due to the smaller group of Negro females.

Why the white females showed trends, and at times significant values, when these were not found in the white males is not clear. It suggests the possibility of some hormonal influence, due to the increase of estrogen in the female during the menopausal period. It is well known that in normal young women cardiovascular complications are infrequent, but the number of cardiovascular complications in the menopausal and postmenopausal females tends to close the numerical difference in the sexes found in the younger age groups. The definite sex difference in human atherosclerosis may be an important clue to the solution of the problem.

One of the most important questions in the treatment of diabetes has not yet been answered. Can the clinician promise fewer cardiovascular complications in the future to nonbrittle diabetics, if they remain under good control? Graph VII shows that patients with good control have slightly lower mean lipid values. This reduction in values is more evident in the male patients, and favors the hypothesis that good control may reduce hyperlipemia and possibly diabetic complications.

This present study was based on differences of mean lipid values between sizeable groups of patients. These differences have to be evaluated carefully when attempting to relate these findings to individual patients. Repeated studies of lipoprotein levels have not been performed at short intervals, in normals or diabetics. The Donner Laboratory in a group of Framingham subjects did some repeated studies at intervals of one and two years, and found many large differences between successive measurements. In a third of the cases, however, the differences for S_f 12-20 values were less than 10 mg. per cent. In the data presented in table 1, the standard deviation of the measures was large. There was, in addition, a small technical error of 5.5 mg. per cent in measuring the S_f 12-20 molecule, and a 5.0 mg. per cent error in measuring the cholesterol from aliquots of the same blood.[14] Thus cholesterol or liproprotein readings with such a wide dispersion would mean little for any individual patient, as the values could be high or low.

One of the objectives of this study was to compare the usefulness of the lipoprotein determinations with that of cholesterol. By reviewing the graphs presented it may be seen that, in general, the S_f 12-20 and S_f 20-100 determinations have no advantage over each other nor over the cholesterol values. The cholesterol determination as a laboratory procedure is one which can be performed without the expensive equipment and well trained staff needed to analyze the serum for lipoproteins.

SUMMARY

1. Nine hundred and one diabetic patients with a mean age of 52.5 years were studied clinically, and had determinations made of their serum cholesterol, S_f 12-20 and S_f 20-100 molecules. It was hoped that these values would give further information pertaining to lipid changes in the complications of diabetes.

2. The mean cholesterol and lipoprotein values of diabetics were compared by dividing the patients into groups, based on the presence or absence of certain diabetic characteristics and complications.

3. The mean lipid values showed some differences and trends. However, a value for cholesterol S_f 12-20, or S_f 20-100 in a single patient cannot be interpreted clinically, since the values of one individual vary greatly from those of another with the same clinical picture.

4. In general the mean S_f values were higher in the white diabetic females in all age groups as compared with white female controls. The white male patients, however, showed no appreciable differences when compared to the controls.

5. The Kimmelstiel-Wilson patients as a group

showed the most consistent elevation of all lipid measures.

6. The white females showed consistent elevation of the means of the three lipid measures with diabetic retinopathy, atherosclerotic complications, hypertension, and vessel calcification. The males, however, did not show any consistency with these characteristics.

7. The males showed a more definite trend of higher lipid values for both poor diabetic control and the taking of insulin. The females showed a lesser elevation in the patients under poor control. This favors the hypothesis that good control may reduce hyperlipemia, and possibly diabetic complications.

8. The Negro females, as compared to the white females, showed similar trends in retinopathy, atherosclerotic complications, and vessel calcifications. In addition, the Negro females showed elevated mean values in both poorly controlled patients and those taking insulin.

9. Arcus senilis was found to be more prevalent in the Negroes than in the whites.

10. In general, the S_f 12-20 and S_f 20-100 values appeared to have little advantage over one another, or over the cholesterol determinations. The technical staff and expensive equipment needed for the S_f determinations make their use impractical for clinical purposes.

SUMMARIO IN INTERLINGUA

Un Studio del Determinationes de Lipoproteina e Cholesterol del Sero in 901 Diabeticos

1. Nove centos e un diabeticos con un etate medie de 52,5 annos esseva studiate clinicamente e se subjiceva a determinationes de lor cholesterol seral, moleculas S_f 12-20 e S_f 20-100. Le spero esseva que iste valores apportarea informationes additional in re le alterationes lipidic in complicationes de diabete.

2. Le valores medie de cholesterol e de lipoproteina in diabeticos esseva comparate per divider le patientes in duo gruppos, super le base del presentia o absentia de certe characteristicas e complicationes de diabete.

3. Le valores medie de lipido revelava certe differentias e tendentias. Tamen, le valor de cholesterol S_1 12-20 o de cholesterol S_f 20-100 in un patiente individual non permitte un interpretation clinic, proque le valores in un date individuo varia grandemente ab le valores in un altere patiente con le mesme tableau clinic.

4. In general, le valores medie de S_f esseva plus alte in diabeticas blanc in omne gruppos de etate studiate que in le nondiabeticas blanc de controlo. Del altere latere, le masculos diabetic blanc non differeva appreciabilemente ab le masculos blanc de controlo.

5. Le patientes de Kimmelstiel-Wilson, reguardate como un gruppo, monstrava le plus uniforme elevation de omne mesurationes lipidic.

6. Le femininas blanc monstrava regular elevationes del valores medie del tres mesurationes lipidic in le presentia de retinopathia diabetic, complicationes atherosclerotic, hypertension, e calcification vascular. Del altere latere, le masculos non revelava ulle correlation con iste characteristicas.

7. Le masculos monstrava un plus definite tendentia verso plus alte valores lipidic tanto sub le conditiones de inadequate regulation diabetic como etiam con le administration de insulina. Quanto al femininas, le patientes a inadequate regulation diabetic monstrava minus alte elevationes. Isto supporta le hypothese que bon regulation reduce hyperlipemia e possibilemente le complicationes diabetic.

8. Le femininas negre, comparate con le blancas, monstrava simile tendentias in le presentia de retinopathia, complicationes atherosclerotic, e calcification vascular. In plus, le negras monstrava elevate nivellos medie sub le conditiones de un regulation inadequate e etiam con le administration de insulina.

9. Arco senil se monstrava plus prevalente in negros que in blancos.

10. In general, le valores de S_f 12-20 e de S_f 20-100 non differeva in lor valor e non se monstrava plus utile que le determinationes de cholesterol. Le facto que determinationes de S_f require un personal technic e costose apparaturas rende lor uso impractic pro objectivos clinic.

ACKNOWLEDGMENT

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Protein Intake

The question whether consumption of excessive amounts of proteins has any harmful effect cannot be answered without qualification. It is well established that Arctic explorers and other persons kept on well-controlled diets, subsisting for many years mainly on meat, do not develop any pathological symptoms. We can therefore conclude that normal adults seem able to tolerate the intake of amounts of protein far above actual requirements. Infants and children, particularly those not adapted to high protein foods, do not do well on diets containing large amounts of protein.[1] It is therefore recommended by several authors that the protein intake be increased only gradually. . . .

The idea that excessive protein intake may lead to development of, or aggravate already existing, hypertension, or that it may cause toxic complications in pregnancy has been abandoned.[2] The assumption that protein food, due to its calorigenic specific dynamic effect, may adversely influence the course of febrile diseases also proved to be false. The consideration that large amounts of protein may damage the kidneys or liver has never been supported by observations in man. Even the exclusion of protein from the diet of people with developed liver or kidney damage is an obsolete practice. More recent studies have shown that diets containing ample amounts of protein very often improve the healing tendency in liver or kidney diseases. Therefore, hypertension, febrile diseases, kidney or liver ailments are no longer considered generally as contraindications to protein rich diets. On the other hand, they should not be regarded as indications for feeding excessive amounts of protein, which has often been suggested with the idea that "enough protein is good, but more may be even better." There is no wisdom in prolonged feeding of diets which contain more protein than 20 per cent of the total caloric intake. Clinical observations suggest, and animal experiments prove, that in certain specific types of liver disturbance feeding of unduly high amounts of proteins may aggravate the disease by overtaxing liver functions involved in utilization of dietary amino acids.[3] It is also self-evident that in kidney diseases where the danger of accumulation of protein end products, increase of blood NPN, is imminent, the protein intake should be restricted or even temporarily eliminated.

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Preliminary Studies on the Demonstration of Insulin in the Islets by the Fluorescent Antibody Technic

Paul E. Lacy, M.D., and Jack Davies, M.D., St. Louis

Since Moloney and Coval[1] have demonstrated that antibodies to insulin can be produced in the guinea pig, it seemed feasible that these antibodies could be labeled with fluorescein isocyanate by the method of Coons and Kaplan[2] and used as an immunochemical stain for insulin. The results obtained from the application of these labeled antibodies to frozen sections of pancreas are reported in this paper.

MATERIAL AND METHODS

The technic as described by Moloney and Coval[1] was used for the production and assay of antibodies to insulin in the guinea pig. Four adult guinea pigs were injected subcutaneously with 25 units of insulin (Iletin) emulsified in a 9:1 mixture of Bayol F and Arlacel A. Two separate injections of this mixture (1.3 ml. each) were given over a period of six weeks. The serum obtained from these animals neutralized the convulsive action in starved mice of at least 0.6 units of insulin per ml. of serum. The maximum neutralizing capacity of the serum was not determined.

The globulin fraction of the immune serum was obtained by precipitation with half-saturated ammonium sulfate and the precipitate was dialyzed against buffered saline (*p*H 7.0) until the dialysate was free of ammonium ions. The protein concentration of this fraction was determined by the method of Weichselbaum.[3] The globulin fraction was conjugated with fluorescein isocyanate by the method described by Coons and Kaplan.[2] In order to remove the nonspecific staining, the labeled protein was absorbed twice with dried, rabbit liver powder (100 mg. per ml.), then centrifuged and filtered.[2]

Pancreatic tissue was obtained from the following animals: guinea pig, rabbit, albino rat, albino mouse, cat and beef. Pancreas obtained at autopsy from a nondiabetic person four hours after death was also used. The tissue was frozen immediately after removal by placing it on dry ice or on a brass block maintained at —25° C. The tissues were cut at five to seven microns in a cryostat.[4] The sections were dried in air at room temperature with the aid of a fan and were used the same day that they were obtained. One drop of the labeled, anti-insulin globulin was placed on the sections for fifteen to twenty minutes at room temperature. The slides were then washed in two changes of buffered saline for ten minutes. After mounting in buffered glycerin, the sections were examined with a dark field microscope using ultraviolet light from a carbon arc source.

The globulin fraction of normal guinea pig serum and anti-egg albumin serum from a rabbit were labeled with fluorescein and used as control solutions.

OBSERVATIONS

Incubation of frozen sections of beef pancreas with labeled anti-insulin globulin produced a bright, yellow-green fluorescence of certain cells in the islets (figure 1). Two types of cells were evident in the islets of the beef pancreas under dark-field illumination with ordinary light. One type contained highly refractile granules while the other type contained less refractile granules (figure 2). The islet cells which were fluorescent corresponded with the cells containing slightly refractile granules (figures 1 and 2). Since the fluorescent cells comprised the majority of the islet cell population, it was assumed that these were beta cells. A positive identification of these cells has not been obtained as yet due to the difficulty of subsequently staining the same section or a contiguous section with aldehyde fuchsin[5] or chrome alum hematoxylin.[6] Paraffin sections of beef pancreas, which had been fixed in Bouin's solution, were stained with aldehyde fuchsin and light green. In these sections, the beta cells were observed to comprise the majority of the islet cell population. The alpha cells were found either as a single row of cells at the periphery of the islet or as small nests of cells within the islets.

A yellow-green fluorescence was produced in the ma-

Presented at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 1, 1957.

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FIG. 1. Fluorescence photomicrograph of a beef islet stained by fluorescent antibody against insulin. The central less refractile cells are strongly fluorescent. X 600.

FIG. 2. Dark field photomicrograph with visible light of the same islet shown in figure 1. Highly refractile cells are shown at the periphery of the islet. X 600.

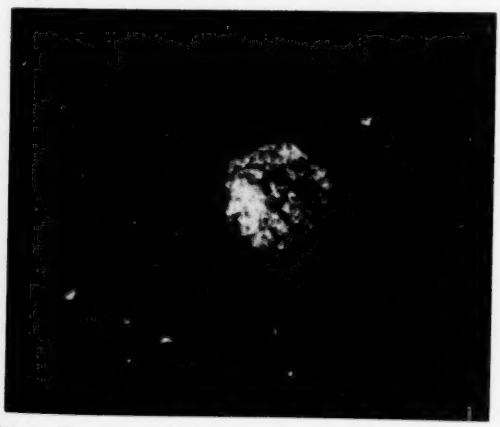

FIG. 3. Fluorescence photomicrograph of an islet of the mouse stained by fluorescent antibody against insulin. X 160.

jority of the islet cells of the cat and apparently in all of the islet cells of the mouse (figure 3) and rat. Fluorescence of the islet cells was not produced in the guinea pig and rabbit pancreas nor in the pancreas of the nondiabetic human subject (table 1). A brownish-red autofluorescence was present in the islets of the human pancreas in both the treated and untreated sections. The presence of this fluorescence made it difficult to be certain of a complete absence of a reaction occurring in the cells. This type of brownish-red fluorescence had been observed previously in the islets of older animals.

The specificity of the induced fluorescence in the islets of the beef and mouse pancreas was indicated in the following experiments (table 2). One unit of insulin (Iletin) added to 1 ml. of labeled, anti-insulin globulin completely inhibited the production of fluorescence in the islet cells. The addition of an equivalent volume of water (*p*H 2.7) to 1 ml. of the labeled globulin did not inhibit the reaction. The reaction was inhibited by the addition of 10 μg. of crystalline pork insulin, which was free of glucagon, to 0.2 ml. of labeled globulin.* Ten μg. of glucagon added to 0.2 ml. of labeled globulin did not inhibit the reaction.

As a control procedure, sections of pancreas from each of the different species were incubated with labeled serum globulin from a normal guinea pig and labeled, anti-egg albumin serum (table 1). The islets could not be distinguished from the faint background fluorescence of the surrounding acinar cells.

DISCUSSION

The production of fluorescence in the majority of the

TABLE 1

Production of fluorescence in islets of different species

Pancreatic tissue	Fluorescence in islets		
	Anti-insulin* globulin	Normal guinea pig globulin	Anti-egg† albumin serum
Beef	Majority of cells	Neg.	Neg.
Cat	Majority of cells	Neg.	Neg.
Mouse	All cells	Neg.	Neg.
Rat	All cells	Neg.	Neg.
Guinea pig	Neg.	Neg.	Neg.
Rabbit	Neg.	Neg.	Neg.
Man	Neg.	Neg.	Neg.

*Globulin fraction of guinea pig serum containing antibodies to commercial insulin (Iletin).
†Rabbit serum containing antibodies to egg albumin.

*The crystalline pork insulin, Lot #499667, and the crystalline glucagon, Lot #258-234B-42, was kindly supplied by Dr. Otto K. Behrens, Eli Lilly and Company, Indianapolis, Indiana. The pork insulin had been treated with trypsin to destroy the glucagon.

TABLE 2

Inhibition of fluorescence in islets of beef and mouse pancreas

Inhibiting agent	Concentration* Units/ml.	μg./ml.	Inhibition
Insulin (Iletin)	1.0		+
Pork insulin†		50.0	+
Glucagon		50.0	—

*Concentration per ml. of labeled, anti-insulin globulin.
†Glucagon-free, crystalline pork zinc-insulin.

islet cells of the beef pancreas, the inhibition of this reaction with insulin and the failure to inhibit the reaction with glucagon indicate that insulin was demonstrated by immunochemical means in the islets of the beef pancreas. The absence of a fluorescent reaction in the islets of the guinea pig, rabbit and human pancreas (table 1) suggests that an immunological difference in endogenous insulin may exist in certain species. Further evidence for a species-difference is provided by the findings of Moloney and Coval[1] that a temporary diabetic state could be produced in mice injected with guinea pig serum containing antibodies to pork insulin but a diabetic state did not occur in the immunized guinea pigs. Further studies are in progress to substantiate these findings by using more purified antigens; crystalline insulin which does not contain glucagon, crystalline beef or pork insulin and glucagon.

The occurrence of fluorescence in all of the islet cells of the mouse and rat pancreas may have been due to the mixed type of antigen that was used since the commercial insulin contained beef insulin, pork insulin and glucagon. The use of more purified antigens may clarify this apparently anomalous situation.

It is possible that the technics outlined in this paper may provide a means of investigating immunologic differences of endogenous insulin in different groups of mammals and may provide the basis for further investigation of those instances in which antigen-antibody reactions may possibly play a role in the pathogenesis of certain forms of diabetes in man.

SUMMARY

The globulin fraction of guinea pig serum, containing antibodies to insulin, was labeled with fluorescein isocyanate. The labeled, anti-insulin globulin was used to stain frozen sections of pancreas from the beef, cat, mouse, rat, guinea pig, rabbit and man. A yellow-green fluorescence was produced in the islets of the beef, cat, rat and mouse pancreas by this labeled globulin fraction. The fluorescent reaction was inhibited in the beef and mouse pancreas by the prior addition of either commercial insulin or crystalline pork insulin to the labeled globulin. The fluorescent reaction was not inhibited by the addition of glucagon. Fluorescence was not produced in the islets of the guinea pig, rabbit and human pancreas.

SUMMARIO IN INTERLINGUA

Studios Preliminar in Re le Demonstration de Insulina in le Insulas per Medio del Technica a Anticorpore Fluorescente

Le fraction globulinic de sero de porcos de India, continente anticorpore a insulina, esseva marcate con isocyanato de fluoresceina. Assi marcate, le globulina anti insulina esseva usate pro colorar sectiones congelate de pancreas de bovo, catto, mus, ratto, porco de India, conilio, e homine. Un fluorescentia jalne-verde esseva producite per le marcate fraction globulinic in le insulas de bovo, catto, ratto, e mus. Le reaction de fluorescentia esseva inhibibile in le pancreas de bovo e mus per le previe addition de insulina commercial o de crystallin insulina porcin al marcate fraction globulinic. Le reaction de fluorescentia non esseva inhibite per le addition de glucagon. Nulle fluorescentia esseva producite in le insulas del pancreas de porco de India, conilio, e homine.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help of Professor W. S. Hartroft of the Department of Pathology and Professor E. W. Dempsey of the Department of Anatomy.

This investigation was aided in part by Grants A-1226 and RG-3784 from the United States Public Health Service.

The Iletin used in this study was obtained from Eli Lilly and Company, Indianapolis, Indiana. Bayol F was obtained from the Penola Oil Company and Arlacel A from the Atlas Powder Company.

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DISCUSSION

DR. WOLF, (*New York*): Have you applied the fluorescent antibody technic to alloxan diabetic rabbits? Since you were unable to differentiate the various cell types in the islets by proper staining technics, it would be interesting to determine the effect of alloxan diabetes on the fluorescence of the islet cells and the time that a change in fluorescence would occur.

PAUL E. LACY, M.D., (*St. Louis*): In studies which are still in progress, we have found that a fluorescent reaction could be produced in the beta cells of the rabbit under conditions which were different from those reported in this study. A few observations have been made on the islets of alloxan diabetic rabbits. The fluorescent reaction was absent from the highly refractile cells (alpha cells) in both normal and diabetic rabbits. The fluorescence was absent or greatly diminished in the remaining less refractile islet cells of the diabetic rabbits.

Hypothalamic Control

The importance of the central nervous system in regulating food intake was discovered through the study of animals with lesions in the hypothalamus. A definite hyperphagia of hypothalamic origin, mentioned briefly by Keller and his associates,[1] was more completely described by Brobeck, Tepperman and Long,[2, 3] who found it to be a primary cause of hypothalamic obesity (Hetherington and Ranson).[4] This hyperphagia was also noted and described by Brooks,[5] Kennedy,[6] Stevenson,[7] Lundbaek,[8] Van Putten,[9] Mayer,[10] Anand,[11] and Larsson.[12] The abnormality may develop almost immediately following the operation, when the animal may appear to be ravenous, "attacking" and "devouring" its food. An intake of two or three times the normal food consumption, persisting at this level for weeks or even months, is not unusual. The animals, whether rats, cats, dogs, or monkeys, become obese to a marked degree.

Discovery of hypothalamic hyperphagia indicated that the nervous mechanisms regulating feeding have at least two distinct components or functions. These are analogous to appetite and satiety, if these words may be used to denote physiological mechanisms rather than subjective states. The hypothalamic lesions inducing hyperphagia presumably interfere with satiety, leaving appetite mechanisms unopposed in their action, thus leading to gross overeating.[13] Anand's observations, however, have shown that the appetite mechanism is also represented in the hypothalamus, more laterally located than the satiety mechanism.[14] By placing small, discrete lesions in all parts of the hypothalamus of cats and rats, Anand confirmed the work of earlier authors regarding the overeating induced by medial lesions in the neighborhood of the ventromedial nuclei bilaterally. But he also noted that lesions in the lateral hypothalamic area brought about a complete failure of feeding, an aphagia. These latter animals did not appear to be abnormal in other respects; they were not somnolent, exhibited no gross changes in behavior or locomotion, and maintained a normal body temperature. (They were not tested in hot or cold environments.) If the operation was designed to produce both medial and lateral lesions, the animals always failed to eat, while animals becoming obese as a result of medial lesions abruptly stopped eating when lateral lesions were created.

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Enzymatic Tests for Glucosuria

John J. Moran, M.D., Paul L. Lewis, M.D.,
John G. Reinhold, Ph.D., and F. D. W. Lukens, M.D.,
Philadelphia

A simple enzymatic test for glucose in urine was recently described independently by Keston,[1] Comeo[2] and by Free.[3] These tests employ paper strips impregnated with glucose oxidase, horse-radish peroxidase and O-tolidine as an indicator. The simplified mechanism of the reaction is as follows:

$$\text{glucose} + \text{glucose oxidase} \rightarrow \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{O-tolidine} \xrightarrow{\text{(peroxidase)}} \text{blue pigment}$$

A urine specimen may be tested for glucose by dipping the impregnated paper strip into the specimen. The strip is then exposed to air for one minute. The presence of glucose is shown by the development of a blue or green color.

It has been known for many years that reagents commonly used to test for glucose in urine react with reducing substances other than glucose. Thus an erroneous diagnosis of diabetes mellitus may be made because of the lack of specificity of the tests. Some of the substances which cause falsely positive reactions are fructose, galactose, arabinose, lactose, maltose, ribose, xylose, uric acid, glucuronic acid, homogentisic acid, salicylates, terramycin,[4] penicillin,[5] thiamine chloride,[6] para-aminosalicylic acid,[7] and streptomycin.[8] Proof that a reducing substance in a sample of urine is glucose may require considerable time and effort. The method of using glucose oxidase appears to be an improved test because of its specificity and simplicity, but insufficient data are available to establish its reliability and relationship to tests now in use. The present study was made to obtain such data.

METHODS

Two commercial preparations utilizing glucose oxidase and a suitable indicator impregnated on paper were studied. Oxidase A was Clinistix made by Ames Company, Inc. and oxidase B was Tes-Tape made by Eli Lilly and Company.

The test strip was immersed in the urine specimen just long enough to wet the paper and was exposed to air for one minute. The development of a blue color in oxidase A or a green color in oxidase B was accepted as evidence of the presence of glucose.

Although product A was not recommended for quantitation, we found that the intensity of the blue color varied with the concentration of glucose. For this reason the degree of color change of the test strip was recorded as faint blue, light blue, or blue.

Product B was stated by the manufacturer to be suitable as a semiquantitative test for urine glucose. The results were recorded as one to four plus according to the color scale supplied by the manufacturer.

In some of the experiments to be described, the color intensities were compared with the results of a semiquantitative copper reduction test. Such tests were done by means of a self-boiling alkaline copper reagent for reducing sugars (Clinitest, Ames Company, Inc.).

In a smaller group of urine specimens, the results of the enzyme tests were compared with those of quantitative measurements of reducing substances. The method employed was the Nelson-Somogyi blood sugar method[9] adapted to urinalysis by our laboratory.

TESTS FOR SPECIFICITY

Mannose, lactose, fructose, xylose, glucuronic acid and arabinose gave no color changes with the oxidase method either in aqueous solution or in urine, but reduced the copper reagent. Sucrose was nonreactive by both methods. Only glucose and galactose reacted with both the oxidase and copper reduction methods. The weak reaction of the latter with glucose oxidase was explained by the presence of glucose as an impurity, since the galactose solution did not react with glucose oxidase after treatment with a washed suspension of baker's yeast.

Albuminuria and hematuria may cause falsely positive tests for sugars in urine when copper reduction methods are used. Therefore the question arose whether the glu-

From the William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia.

cose oxidase test would be so affected. In a series of 223 urine specimens selected at random and showing negative tests for protein, 217 showed no evidence of glucose while six gave positive reactions when tested with oxidase A. The incidence was nearly identical in a similar series of 221 specimens in which albumin was present, obtained from the same population of patients as the preceding. Of these 214 were negative for glucose while seven were positive. If albumin affected the glucose oxidase test, it would appear that a greater frequency of positive tests should have occurred in the latter group. Since there was no difference it would appear that albuminuria has no effect.

Seven urine specimens showing gross hematuria gave negative tests for glucose with the oxidase and copper reduction methods. Thus hematuria does not interfere with the glucose oxidase test.

COMPARISON OF THE ENZYMATIC AND COPPER REDUCTION METHODS FOR DETECTION OF GLUCOSURIA

A total of 2,002 urine specimens were tested by means of glucose oxidase strips and the semiquantitative copper reduction method. Of this total, 1,493 were obtained from diabetic outpatients and 509 from unselected hospitalized patients.

In the diabetic group (figure 1) both oxidase tests and copper reduction tests were negative in 56 per cent of specimens with oxidase A and 49 per cent with oxidase B. Both oxidase and the copper reduction tests were positive in 32 per cent with oxidase A and 35 per cent with oxidase B. Thus the results agreed in a total of 88 per cent with oxidase A and 84 per cent with oxidase B.

In the urine specimens from hospitalized patients (figure 2), both oxidase tests and copper reduction were negative in 90 per cent of specimens with oxidase A and 96 per cent with oxidase B. Both oxidase and copper reduction tests were positive in 5.1 per cent of specimens tested with oxidase A and 1.5 per cent with oxidase B. The agreement between enzymatic and copper reduction tests was somewhat higher than in the diabetic group, being 95.1 per cent with oxidase A and 97.5 per cent with oxidase B.

Disagreement was most frequent in the diabetic patients with positive oxidase reactions and negative copper reduction tests. This occurred in 9.1 per cent of specimens tested with oxidase A and 12 per cent of those tested with oxidase B. The corresponding figures for the hospitalized group were 2.8 per cent with oxidase

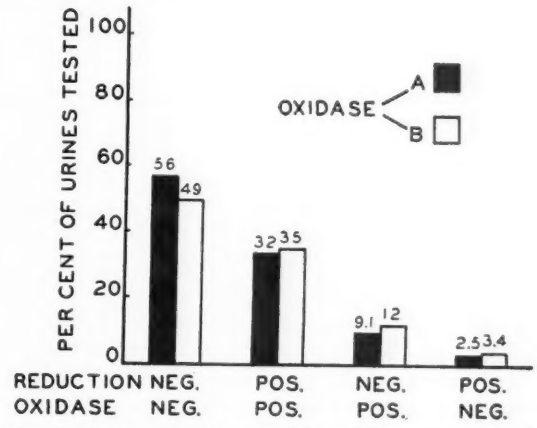

FIG. 1. Copper reduction method for sugar in urine compared with glucose oxidase test strips. One thousand, four hundred and ninety-three urines were tested with oxidase A and 655 with oxidase B. All were from diabetic outpatients.

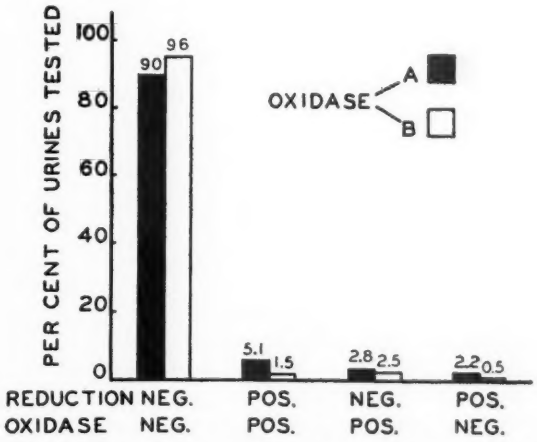

FIG. 2. Copper reduction method for sugar in urine compared with glucose oxidase test strips. Five hundred and nine urines were tested with oxidase A and 198 with oxidase B. All were selected at random from hospitalized patients.

A and 2.5 per cent with oxidase B. The reason for the occurrence of a positive oxidase test and a negative copper reduction test is the greater sensitivity of the oxidase reaction.

In the diabetic group the oxidase test was negative and the copper reduction test was positive in 2.5 per cent of urine specimens tested with oxidase A and 3.4 per cent tested with oxidase B. In the group of urine specimens from the hospitalized patients the corresponding figures were 2.2 per cent and 0.5 per cent with oxidase A and B respectively. Reducing substances other than glucose would account for these results.

EVALUATION OF GLUCOSE OXIDASE STRIP PREPARATIONS FOR MEASUREMENT OF GLUCOSE CONCENTRATION

Figures 3 and 4 show comparisons of the intensity of the color reaction of the glucose oxidase test strips with the quantitative measurement of sugar in urine. One hundred and seventeen urines were tested with oxidase A and 107 with oxidase B. These specimens were obtained from diabetic outpatients.

Although there is a general correlation between the glucose concentration in urine and the intensity of the glucose oxidase tests, the gradations in color do not sharply distinguish between the different concentrations of glucose. A number of weakly positive tests with both enzyme preparations were associated with urine glucose concentrations of less than 0.1 per cent, and a few scattered specimens gave strongly positive tests with glucose concentrations in this range. A preponderance of the more strongly positive tests occurred in the urine containing more than 0.4 per cent of glucose. The absence of any negative reaction in urines having more than 0.2 per cent glucose indicates that such a response excludes clinically significant glucosuria.

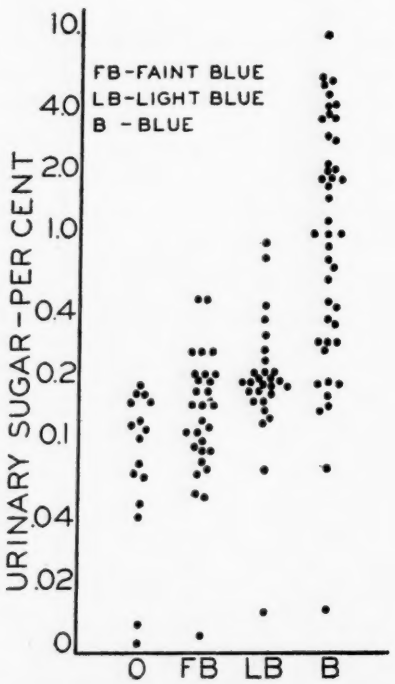

FIG. 3. This relates the tests with oxidase A to the quantitative determination of reducing substances from diabetic outpatients. Concentrations of glucose in urine are shown on the logarithmic scale. The lower cycle shows concentrations of reducing substance up to 0.1 per cent, the center cycle from 0.1 tc 1.0 per cent, and the upper cycle from 1.0 to 10.0 per cent.

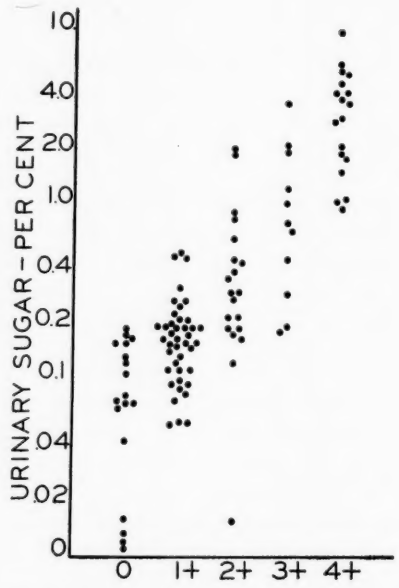

FIG. 4. This relates the tests with oxidase B to the quantitative determination of reducing substance in 107 urines from diabetic outpatients. See figure 3 for explanation.

The wide range of glucose concentrations associated with the production of a given color intensity shows that glucose oxidase test papers have distinctly limited usefulness for the semiquantitative measurement of glucose concentrations in urine. One exception in which the grading of the color intensity was helpful is for detecting glucose concentrations of 1 per cent or greater in the urine of known diabetics. All urine specimens containing 1 per cent or more of glucose gave a blue reaction with oxidase A. The detection of urine sugar of 1 per cent or higher is considered by the Diabetes Clinic of this hospital to be important in the control of diabetes mellitus. A less intense color reaction, therefore, could be accepted as evidence that the specimen contained less than 1 per cent glucose. However, it should be noted that a blue reaction often occurred when less than 1 per cent glucose was present.

Oxidase B may be used in a similar way since reactions of 2+ and higher were found in all urine specimens in which the glucose concentration exceeded 1 per cent.

Unless the user takes into account the much greater sensitivity of these glucose oxidase tests as compared with the older methods, misleading conclusions may be

drawn concerning positive tests. The color reactions both of oxidase B and of the copper reduction methods are graded as to one to four plus. However, on the label the approximate range of a one-plus reading with oxidase B is "about 0.1 per cent," while with a copper reduction one plus represents 0.5 to 1.0 per cent. This difference in range applies throughout the scale, except for the four-plus reading (above 2 per cent). Although the range is clearly stated by the manufacturer of oxidase B, confusion could result, particularly in the minds of the patients.

APPLICATION OF THE ENZYMATIC TESTS TO URINALYSIS

Glucose oxidase used in the form of test papers for the detection of glucosuria has great advantages over older methods in simplicity and specificity. It is well adapted to rapid screening of routine urines for glucose. When so used, the finding of a positive test should be supplemented by a quantitative measurement by a dependable method to establish the amount of sugar if this information is required.

Glucose oxidase offers a most convenient and valuable means of ruling out falsely positive copper reduction tests due to reducing substances other than glucose. For this purpose it is superior to the older technics depending upon fermentation with yeast, formation of osazones, or paper chromatography.

The oxidase method has been in use in the Diabetes Clinic of this hospital for six months. For the first few weeks, the results were compared with those of the copper reduction method. It soon became obvious that although there might be differences in single samples of urine, the total number of sugar-free samples per clinic day was the same by both methods. This has led to the conclusion that the greater sensitivity of the oxidase method does not interfere with its practical use in observing and maintaining freedom from glucosuria provided the light blue and faint blue color reactions are considered negative.

It appears that glucose oxidase test strips will be useful for two purposes: (a) for the diagnosis of diabetes in which the ease of the method will facilitate screening in doctor's office or health center and (b) in the control of diabetes, especially the mild (i.e. stable) form, in which freedom from glycosuria can be observed with this test. The greatest difficulty in introducing this test in our clinic was the campaign of education needed to eliminate the traditional one to four plus recording of results. Unpublished data indicate that these are too erratic to justify the attention they have received in the past.

SUMMARY

The results of 2,002 oxidase tests of urine glucose were compared with those of copper reduction methods. Of these, 1,493 were from diabetic outpatients and 509 were random urine samples from hospitalized patients. The results of the oxidase and copper reduction methods agreed in 85 to 88 per cent of the diabetic group and in 95 to 97 per cent of the hospital group. The discrepancies in the diabetic group were mainly a result of the greater sensitivity of the oxidase tests.

Glucose oxidase papers did not accurately measure concentrations of glucose in urine, although some information of clinical value, especially concerning the occurrence of higher concentrations, i.e., 1 per cent of glucose or greater, could be obtained by grading the intensity of the color formed.

SUMMARIO IN INTERLINGUA

Probas Enzymatic Pro Glucosuria

Le resultados de 2.002 probas a oxydase urinari esseva comparate con le resultados de methodos a reduction per cupro. Le serie total del specimens includeva 1.493 ab diabetic patientes visitante; 509 esseva specimens urinari ab non seligite patientes hospitalisate. Le resultatos del methodos a oxydase e a reduction per cupro esseva conforme in 85 a 88 pro cento del gruppo diabetic e in 95 a 97 pro cento del gruppo hospitalisate. Le discrepantias in le gruppo diabetic reflecte principalmente un plus alte grado de sensibilitate in le proba a oxydase.

Papiros a oxydase de glucosa non mesurava accuratemente le concentrationes de glucosa in le urina, sed per graduar la intensitate del resultante color il esseva possibile obtener certe informationes de valor clinic, specialmente in re le occurrentia de plus alte concentrationes de glucosa—i.e., concentrationes de 1 pro cento o plus.

ACKNOWLEDGMENT

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Essential Fatty Acids

Although the exact and optimal requirements for fat in the diet have not been established precisely, it has been demonstrated that in the complete absence of dietary fat, normal growth and well-being are not possible. In 1929, Burr and Burr reported that rats on fat-free diets failed to grow normally and developed a scaliness of the skin and tail. Kidney lesions were also present; in the female ovulation was irregular and male rats on a fat-free diet could not be induced to mate. The syndrome which develops could be completely alleviated and the animals restored to normal health by the administration of linoleic acid ($C_{17}H_{31}COOH$). No other fatty acid tested was curative for the fat-deficiency, and high-grade butter fat was also ineffective, thus supporting the view that the fat-soluble vitamins were not concerned with the development of the deficiency condition. A few years later the same laboratory reported that linolenic acid ($C_{17}H_{29}COOH$) was also effective in alleviating the fat-deficiency manifestations. It was suggested that the capacity of the animal organism to synthesize certain unsaturated fatty acids is limited and that these fatty acids, e.g., linoleic and linolenic acids, therefore, may be considered to be essential fatty acids in that they must be supplied in the diet.

The results of Burr and Burr were confirmed in several laboratories and the concept of essential fatty acids extended to include the C_{20}, four double bond fatty acid, arachidonic acid ($C_{19}H_{31}COOH$). Turpeinen and Smedley-MacLean and her associates demonstrated that arachidonic acid was effective in curing the fat-deficiency disease in rats and, indeed, that this fatty acid was superior to the other essential fatty acids in curative power. Arachidonic acid, administered as the methyl ester, was found to be more active than methyl linoleate when judged by weight increase of the experimental animals. The two esters were approximately of equal potency with respect to ability to cure skin lesions. Smedley-MacLean and her colleagues concluded that arachidonic acid is necessary for new tissue synthesis. In circumstances of diminished dietary intake, its concentration in the subcutaneous tissue is depleted as a result of supplying the rapidly growing parts.

A state of chronic essential fatty acid deficiency has been produced in adult mice without the outward symptoms of acute deficiency, except for growth inhibition. In these animals, stress conditions, such as injury, pregnancy and x-irradiation precipitated the animals into the acute deficiency state. Growth inhibition due to essential fatty acid deficiency is not modified by hypophyseal growth hormone injection. Symptoms of essential fatty acid deficiency have also been described in dogs. Puppies on a low-fat diet developed a generalized flaky desquamation with coarse dry hair at about three months of age. These skin changes were prevented by the inclusion of lard in the diet.

Dogs on a low fat diet (1 per cent of the calories as fat) showed skin lesions and a lowered content of conjugated unsaturated fatty acids in the serum and skin.

At the present time too little is known about the requirement of man for unsaturated fatty acids. Two infants maintained by vonGröer on a diet very low in fat grew quite well, but one developed a generalized eczema. Holt and his colleagues reported that one of three infants kept on a fat-free diet developed an eczema which was cured by feeding fat. A distinctly lower than normal blood content of both arachidonic and linoleic acid was found in infants with uncomplicated eczema. Some of the children showed remarkable clinical improvement after lard had been added to the diet. Similar findings were reported from another clinic. The data with human subjects are not as uniform as with experimental animals. This may be due to uncontrolled variables or species differences.

From the book *Diseases of Metabolism* edited by Garfield G. Duncan, M.D., published by W. B. Saunders Co., Philadelphia, 3rd ed., 1952, Section "Lipid Metabolism" by Abraham White, Ph.D., pp. 198-99.

EDITORIALS

THE RESPONSES OF THE SMALLER BLOOD VESSELS AND THE SERUM PROTEINS IN PREGNANT DIABETIC SUBJECTS

Conjunctival vessels have been under scrutiny for many years. They have been studied anatomically in an effort to learn more of the role of the capillaries in human disease. The conjunctiva represents a unique area of the body in which it is possible to study and trace the capillary bed from the arteriolar to the venular end. This observation can be made with a minimum of trauma so that the normal activity of the functioning components of the bed will not be disturbed. Recent anatomical studies have been reported by Grafflin and Corddry (1953), Bloch (1954) and Meighan (1956).

Bloch (1956) believes that some of the changes which take place in other small vessels of the body are similar to those which are observed in the conjunctival vessels.

The conjunctival vessels have been studied in patients with systemic hypertension (Lee and co-workers, 1951), (Glack and co-workers, 1949). Lee (1955), for example, noted changes in hypertension associated with vasoconstriction, abnormal reactivity to locally instilled epinephrine and permanent change in vascular topography in the form of elongation, coiling and tortuosity of the tissue capillaries. He also described vasoconstriction of the minute terminal arterioles and metarterioles.

According to earlier workers, Rollin (1934), Pletnava (1938) and Sedan-Bauby (1949), the condition of the conjunctival vessels is a reliable guide to the state of the circulatory system in individuals with systemic hypertension.

The conjunctival vessels in diabetics have been under close study for many years. The first reference to the occurrence of aneurysmal dilatations in the conjunctival vessels in diabetes is that of Bajardi (1901). He stressed their significance only when they were present in large numbers. A similar conclusion was reached by Keller in 1920 and Streiff in 1914. Keller (1920) also noted the presence of arteriolar constriction, venular dilatation and congestion of the conjunctival vessels. Ruedeman (1933) found no diagnostic changes in the diabetic conjunctiva and Waite and Beetham (1933) reported the results of an examination with a slit lamp of the conjunctiva of over 2,000 diabetic subjects and made no mention of microaneurysms. Koby (1930) noted considerable dilatation of the conjunctival veins in diabetics. Along the same line, Janert and Olbert (1955) found changes in the conjunctival veins which were significantly more frequent in diabetics than in normal subjects.

Ashton (1949) made preparations of post-mortem conjunctiva and found no evidence of microaneurysms while Friedenwald in 1952 concluded that about 5 per cent of diabetics with retinopathy showed saccular aneurysms in the conjunctival capillaries, an incidence which he considered to be somewhat higher than in nondiabetics. McCulloch and Pashby (1950) noted a very high incidence of conjunctival aneurysms in diabetics as did Weinstein and Forgacs (1951).

Conjunctival aneurysms do occur occasionally in healthy subjects but more frequently with advancing age in otherwise healthy elderly people. They have been described in many other nondiabetic diseases including syphilis, anemia, leukemia, renal disease and gout and particularly in patients with marked arteriosclerosis.

Cook reinvestigated this problem in 1954 and noted an increased incidence of saccular conjunctival aneurysms in the hypertensive patients in the diabetic group as compared to those without hypertension. He believes that the greater incidence of aneurysms in the diabetic as compared to the nondiabetic subjects may be explained by the greater incidence of hypertension in the diabetic group. Therefore he did not believe that one could postulate a specific conjunctival vascular lesion in diabetes analogous to that occurring in the retina.

Hoffmann and Kruger (1955) applied suction in measured quantity to the bulbar conjunctiva and found variations in capillary fragility, particularly in patients with hypertension. An astonishingly low abnormality of conjunctival capillary fragility in patients with diabetes was reported.

Ditzel and Moinat (see pages 307-23) have reinvestigated the changes which may take place in conjunctival vessels in normal patients during pregnancy and compared those with the alterations which occur in diabetics going through pregnancy. These investigators were aware of the earlier work of Landesman and his co-workers (1953, 1954) who had reported progressive constriction of the arterioles, reduction in blood flow, during pregnancy in normal individuals, particularly in the third

trimester. They were also stimulated to carry out this investigation by the earlier reports of Odell and his coworkers (1947) who had attempted to correlate intravascular erythrocyte aggregation as seen in the conjunctival vessels with changes in plasma protein which had been shown to occur during pregnancy. Ditzel and Moinat's studies are of particular interest because they show a definite association in protein changes during pregnancy and vascular and intravascular responses of the conjunctival vascular bed. Ditzel and Moinat observed the lateral bulbar conjunctiva with a stereo binocular ophthalmoscope. Usually they employed a magnification of forty-eight to sixty-four times. Occasionally, for short observations, they used a magnification of 144 times. The vessels were photographed, the speed of the linear velocity of the blood cells and the diameter of the vessels were measured. They graded the changes in the caliber of the arterioles, capillaries and venules and also observed intravascular erythrocyte aggregation as well as perivascular changes.

All of the alterations which Ditzel and Moinat observed in diabetics during pregnancy occurred also in normal pregnancies uncomplicated by diabetes. However, the changes took place earlier and with greater frequency in the diabetics than in the nondiabetics. The changes they noted included arteriolar constriction, venular distention, capillary tortuosity, edema, hyaline infiltration and erythrocyte aggregation. In normal pregnancy there was a definite alteration in blood proteins characterized by lowering of the total protein content, a progressive decrease of albumen and a slight increase in alpha-2 globulin. The most consistent change noted in the globulins appeared to be an increase in the beta lipoproteins during the last weeks of pregnancy. The same changes took place in the diabetic pregnancy, but the major fluctuations in the serum protein pattern occurred earlier in the pregnancy. Lipoproteins were involved particularly. These changes may have been related to the periods of poor control of the diabetes. After delivery, the different factions tended to return to their prepregnant level.

These results are quite stimulating. They may be related to altered hormone equilibrium occurring in pregnancy. It is possible that the conjunctival vessel changes may be reflecting alterations that are occurring in vessels of similar size elsewhere in the body. It may be that the vasomotor changes observed in the conjunctiva are the initial step leading to vascular degeneration in the vessels of similar size throughout the body.

Although earlier workers had observed an increase in severity of diabetic retinopathy during pregnancy, in the present series Ditzel and Moinat were unable to detect any significant increase in retinopathy associated with changes observed in the conjunctiva. It may be that the conjunctival changes are earlier ones and similar alterations cannot be picked up in the retina due to the lack of adequate magnification by the presently available methods for studying the retinal vessels and periretinal vessel tissue.

It may be that the vascular changes noted in the conjunctival vessels in pregnant diabetics are closely related to the protein changes studied. Possibly they are related to some other factors such as alterations in systemic blood pressure. Regardless, the technic employed by Dr. Ditzel and Dr. Moinat and their predecessors affords an opportunity to study the predisposing and disposing factors for the vascular degenerations that are responsible for some of the most serious complications of diabetes.

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MECHANICAL FACTORS IN THE LOCALIZATION OF ATHEROSCLEROSIS

No single cause has been found which adequately explains the complexity of the atherosclerotic process. Attention has been given to possible etiologic factors such as: race, heredity, age, sex, climate, diet, living standard, occupation, emotional stress, composition of the blood and mechanical factors. The demonstrable predilection of atherosclerotic plaques for certain fairly well-defined areas of the arterial system has given rise to many theories to explain this localization. The present paper proposes to review current progress in this area.

Among the regions with high incidence of arterial lesions are: the left middle cerebral, the left coronary, the splenic, and the aortic at four levels (at the coronary orifices, at the intercostal orifices, at the lumbar orifices and at the bifurcation).

A widely accepted explanation of the localization of tissue change is derived from its occurrence in regions subjected to extremes of pressure and stress. Leary[1] noted that the ascending aorta is momentarily subjected to greater stress than any other part of the arterial system with the closure of the aortic cusps. The pressure is then generalized and reduced by the elastic aorta. Leary believes that the stress on the proximal portions of the coronary arteries probably accounts for the apparently selective frequency of coronary sclerosis, though the systolic pressure occurring while their muscular branches are compressed is also an important factor. In his comprehensive review of the literature on arteriosclerosis in 1944, Hueper[2] cites other stress and strain hypotheses, such as the impact of the pulse wave causing a longitudinal and transverse stress while the action of the blood current creates oblique pressure. These antagonistic forces are supposed to cause primary intimal sclerosis. Sudden and marked fluctuations in pulse pressure have been suggsted as causing vascular tortuosities by producing excessive longitudinal tensions in the vascular wall.

Regions of arterial branching are frequent sites of pathologic change. Winternitz[3] has noted the frequency of intramural intimal circulation which anastomoses with the adventitial vasa in such areas. He showed that this development is accelerated in atherosclerosis. Duguid[4] suggested that the branches of a vessel drag on the main vessels during systolic lengthening causing splits and chinks and separation of the intima from the media.

In a recent article J. B. Duguid and W. B. Robertson[5] emphasize that the mechanical factors concerned in arterial function must be taken into account in the search for the genesis of atherosclerosis. The arteries are elastic tubes with muscle, elastic tissue and fibrous tissue. The arteries dilate with each pulse beat; the recoil is a function of the three elastic tissues, but the muscle tissue is mainly responsible for the contraction. These authors maintain that the muscle of the arterial wall has a greater degree of elasticity than the elastic and fibrous tissue cells. Impairment of the flexibility of the intima leads to the kind of structural disorganization seen in atherosclerosis.

Since thrombi of all sizes and kinds occur with astonishing frequency in the intimal and subintimal tissues, repair produces a fibrous overgrowth within the wall which interferes with ability to recoil. Cholesterol and other fatty debris are products of blood destruction. These can be accounted for by small hemorrhages recurring over long periods.

These authors insist that movement is the crucial factor in the cause of atherosclerosis. They agree with Harrison who has shown that cholesterol is merely an effective "marker" of structural damage. This interpretation likewise supports that of Aschoff, who believed that fat concentration in the food does not cause atheroma but modi-

fies the patches by determining the presence or absence of fatty change.

Points of fixation and surrounding tissue pressure have been variously interpreted as causative and inhibiting factors. Moschcowitz[5a] postulates a relation between impaired expansile mobility of vessels and the localization of lesions. His examples of such areas include: the intercostal vessels which fix the posterior wall of the aorta, sections of the abdominal aorta fixed against the rigid vertebral column, the portions of the dural vessels which lie within the bony framework, that portion of the carotid artery which traverses the canal in the temporal bone and along the cavernous sinus, the left coronary artery which is imbedded in firmer muscular wall than the right, and retinal vessels which are enveloped by the tension of the eyeball.

Other instances have been cited in which firm support of the arterial wall is credited with reducing the incidence of lesions. The internal carotid artery in the base of the skull is very rarely affected by atherosclerosis while the portions lying proximally and distally are notoriously prone to involvement (Hultquist[6] cited by Hueper[2] and Willis[7]). The portion of the anterior descending coronary artery which lies buried in the myocardium is spared from atherosclerosis while branches of the same artery lying in epicardial fat, and therefore not surrounded by appreciable tissue pressure, are affected by atherosclerosis (Geringer[8] cited by Willis[7]).

It becomes obvious that if these mechanical theories of causation are to be properly evaluated, mathematical means of calculating pressures at various points in the arterial system must be developed. This in turn would seem to be contingent upon the nature of the fluid transmitting the pressure and the characteristics of its flow.

Some of the fundamentals involved in any such systematization are presented by L. E. Bayliss[9] in his chapter on rheology of blood and lymph in *Deformation and Flow in Biological Systems* by A. Frey-Wyssling in 1952. Bayliss reminds us of Poiseuille's work in 1836. The variable factor of viscosity has required certain modifications in Poiseuille's law but in cases where the sheer stress is strong enough and in vessels with a radius greater than .01 cm., the flow obeys the law. This early law, which relates the amount of flow proportionately to the fourth power of the radius of the tube, contributes materially to an understanding of how the smallest arterioles and capillaries are able to withstand the pressures to which they are subjected and perhaps thereby to their ability to remain free of atherosclerotic manifestations.

Bayliss's chapter describes enough of the variable factors requiring compensatory calculations to justify his conclusion that "the whole problem of pressure-flow relation of blood needs much further investigation." Even sharper doubts are raised more recently by Jeffords[10] who attributes a cone-shaped form to the arteries instead of the cylindrical shape upon which Poiseuille's equation is based. The fact that blood is not a simple Newtonian or viscous fluid is also counted against the applicability of Poiseuille's equations.

Another pioneer in the reduction of physiological components to measurable terms is R. H. Woods[11] who applied the classical law of Laplace to hemodynamics in 1892. This law's applicability has recently been developed further by A. C. Burton.[12] The total tension in the blood vessel wall is given by Laplace's law as the hydrostatic pressure within it multiplied by the radius. Since the radius changes by a factor of 10,000 times as we pass from the aorta to the capillaries, its importance in determining the maintenance tension within the wall is apparent. Burton correlates the amount of elastic tension in the vessel wall to the amount of elastic tension required to hold the wall of the vessels against prevailing hydrostatic pressure of the blood. He sees the elastic tension in the vessel wall as the factor which makes possible the establishment of an equilibrium between active tension supplied by smooth muscle fiber and the pressure generated within the vessel. A third component of total tension suggested by Burton is the interfacial tension which could result from friction between the vessel wall and the fluid flowing through it.

Elastic diagrams of the aorta and the vena cava are produced by Burton by plotting the tension as the ordinate against the radius (or circumference) of the vessel using data on volumes at different pressures and Laplace's law. A further suggested application of Laplace's law is the calculation of the critical closing pressure of vessels as the active tension in dynes per centimeter divided by the unstretched radius of the vessel.

The application of Burton's development of Laplace's law to curved and branching vessels has been suggested by G. C. Willis[7] with the following modification of the law to meet these conditions:

$$T = \frac{P}{\frac{1}{R} + \frac{1}{r}}$$

(Where T is tension in dynes per centimeter, P is

excessive hydrostatic pressure inside the vessel over and above local surrounding tissue pressure in dynes per square centimeter, R is the radius of the arc made by a curved vessel in centimeters, and r is the radius of the vessel in centimeters.) Willis suggests that the calculation of increased tension T at various sites under experimental conditions, vascular anomalies, and in the ordinary atherosclerotic subject corresponds remarkably well with the localization of atherosclerosis.

Willis discusses the relation of the factors influencing the operation of this formula with respect to the mechanical factors influencing the localization of atherosclerotic plaques. (1) *Blood pressure within the artery.* Sites of localized hypertension have a high incidence of atherosclerosis. (2) *Surrounding tissue pressure.* Arterial segments supported by surrounding tissue pressure are sometimes spared. (3) *Radius of the artery.* Increase in radius resulting from dilation increases tension in the wall facilitating atherosclerosis. (4) *Curving of the artery.* The elongation of the outer curves of such arteries, occurring simultaneously with tangential stretching of the inner curves during the recoil following the cardiac systole, may account for the frequency of atherosclerosis in curved arterial segments.

Rodbard and co-workers[13] have studied the relationship of diameter of the vessel and stretching force to dilation. By measuring the force required to balloon segments of Penrose tubing under varying conditions they claim to have confirmed the principles set forth above by Burton. It is recognized by these investigators that their model departs in many ways from the situation which holds for blood vessels but confirms the fact that external support inhibits dilation and that increased radius caused by stretching facilitates further dilation.

Evidence which would seem to contradict the validity of the Penrose tube findings was compiled by Roy[14] whose tests with aortic segments showed that the degree of distensibility decreased in proportion to the weight applied. The studies of Roy and others on the elastic properties of different tissues and possible relationships of elasticity to structure and function are reviewed in another article by Burton.[15] He compares the varying structure of blood vessel walls in different parts of the body. Graduations of elasticity or distensibility appear to be achieved by variations in the proportions of endothelial cells, elastic tissue, smooth muscle, and collagenous fibers. This variety recommends a cautious use of any formulae designed to apply to all parts of the vascular system.

Discussion. Just as there are widely conflicting theories concerning the pathogenesis of atherosclerosis, there are divergent opinions concerning the importance of the mechanical factors and the methods of measuring them. Recent developments in the fields of biophysics have led to a rediscovery and re-evaluation of the laws and formulae of such physicists as Poiseuille, Laplace, and Hooke. The applicability of their theories to conditions within the walls of living arteries still remains to be convincingly demonstrated. Under normal conditions much still remains to be learned concerning the influence of the viscosity of the blood, the relation of actual shape of the blood vessel to the cone or the cylinder, the effect of the varying relation of potential and kinetic energy in various parts of the arterial system, and varieties of morphology of the blood vessel itself in different parts of the body. Pathologic conditions would introduce new factors including changes in the viscosity of the blood and muscle tone.

In spite of these imponderables and others yet undiscovered the high mortality from cardiovascular disorders places increasing urgency upon further research in this field. The demonstrable patterns of localization of the atherosclerotic process especially under hypertensive conditions suggest that the accumulation of further experimental and clinical evidence in confirmation or contradiction of mathematical laws and formulae will be highly desirable. Accumulations of experimental evidence may eventually lead to the discovery of new laws which could contribute materially to our understanding of the atherosclerotic process.

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IDIOPATHIC HYPOGLYCEMOSIS IN CHILDREN AND CENTRAL NERVOUS SYSTEM DAMAGE

McQuarrie and co-workers,[1, 2] in their reports of 1949 and 1953, called attention to a syndrome noted in children consisting of hypoglycemic episodes and convulsions. While they reported on thirty-eight cases with episodes of spontaneous hypoglycemia from various causes, the most fascinating of the group were twenty-five children with the condition that these authors referred to as "idiopathic hypoglycemosis." Investigation of this group of children revealed no specific organ, system or hormonal etiology, but the children were found to have increased sensitivity to injected insulin or impairment of the normal response to hypoglycemia. Some children had undergone partial or subtotal pancreatectomy without relief of symptoms. Nor did the histologic examination of the pancreas provide a clue to the disorder. An alpha cell abnormality, once suspected to be the etiologic factor, has as yet to be proved.

Eleven of the twenty-five patients included as idiopathic hypoglycemics showed a familial or hereditary factor in the etiology. Twenty-one of the twenty-five patients had the onset of clinical hypoglycemia under two years of age. There were eighteen boys and seven girls in the group. McQuarrie and co-workers stated that they saw only children who had the severest conditions, and undoubtedly a far greater number of children who have the less severe type are never diagnosed properly, recover spontaneously, or automatically adjust their own diets by voluntary food selection.

The presenting complaint in nineteen cases was convulsions, coma with convulsions in three, and staring, pallor, strabismus, tremulousness, and so forth in three. Fasting blood sugar levels as low as 10 to 15 mg. per 100 ml. of blood (macro) were noted. On corticotropin, 10 mg. every six hours, the fasting blood sugar levels could be maintained in the range of 50 to 90 mg. per 100 ml. and convulsions could be prevented. The nondiabetic type of curve was noted on the epinephrine and glucose tolerance tests.

While mental retardation and obvious brain damage were present in many of McQuarrie's patients, there were a few who were normal and later normoglycemic without treatment. In some instances the severely damaged infants were known to have developed normally before the onset of the convulsions.

Gall and Burke[3] reported two additional cases of hypoglycemosis, one in which the patient was a four and a half-month-old boy in whom a severe degree of cortical atrophy was demonstrated, and a second in which the patient was a severely damaged eight-month-old child in whom convulsions had been noted from the second day of life.

The beneficial effects of corticotropin therapy did not appear to be temporary in some of McQuarrie's cases. Of the cases reported by Gall and Burke, one child succumbed following progressive deterioration and convulsions at four and a half years of age, while the other child was severely damaged mentally though continually receiving corticotropin therapy.

All of the authors cited above call attention to the importance of obtaining fasting blood sugars in infants having convulsions, because salvation from severe damage of the central nervous system could be effected by corticotropin therapy. It is apropos to mention dangers to the brain of hypoglycemic episodes from overdosage of insulin inasmuch as resultant damage to the brain would be the same.

Though no protocols have been published in any of these above-cited cases many authors have demonstrated changes in the brain consequent to insulin shock therapy or hypoglycemic episodes.

Baker[4] in 1939 subjected rabbits to repeated insulin shock insults and killed the animals at various time intervals during the study. He noted that nerve cell damage did occur, but was by no means as striking as cerebral hemorrhage, areas of demyelinization, encephalomalacia and glial reaction. The changes were thought likely to be only temporary in the brains of animals subjected to one or several hypoglycemic episodes. Baker suggested that cerebral damage in hypo-

glycemia might be due to qualitative circulatory disturbance, inasmuch as blood reaching the brain is deficient in the proper nutritive materials.

Heberden and Friedlander[5] in 1955 reported pathologic changes in the brain of a two-year-old diabetic child who had suffered from prolonged overdosage with insulin and who finally succumbed to convulsions many months later. In this same child ventricular dilatation and cortical atrophy were suspected following neurologic, electro-encephalographic and pneumo-encephalographic studies.

Numerous authors were cited by Heberden and Friedlander in a discussion of the histopathologic changes in the brain associated with recurrent and prolonged hypoglycemic episodes. Gross vascular lesions, complete necrosis of nerve cells, and encephalomalacia of the cortex, thalamus, and caudate and lenticular nuclei occurred. Lesions of the basal ganglia similar to effects of severe anoxia, carbon monoxide poisoning, cardiac arrest and status epilepticus have also been described as neuropathologic changes in hypoglycemic deaths.

Heberden and Friedlander concluded that coma up to three hours can be associated with a lack of demonstrable brain damage and that the level of blood sugar seems to bear little relationship to the duration of coma. A progressive rise of the blood sugar is not necessarily accompanied by a return to consciousness.

In diabetics, use of the long-acting insulins has, in a sense, added a further threat of hypoglycemic coma because of the insidious approach of the coma and the sustained action of the agent.

Experimentally, hypothermia and barbiturates have been noted to protect animal brains from hypoglycemic damage. Cortisone and corticotropin have been recommended to alleviate the prolonged hypoglycemia.

It is generally accepted that nerve cells require glucose for oxidative processes and that the cells themselves have meager glucose reserves. Hence, the cells depend on blood glucose to a large extent.

Those of us treating children, therefore, must be particularly alert to the hazards of hypoglycemic episodes, whether from administration of insulin or from other causes, because the immature central nervous system is more susceptible to convulsions and perhaps to permanent brain damage than the mature central nervous system.

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Max Rubner
1854-1932

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Few people have made contributions to the field of metabolism comparable to those of Max Rubner. His development of the Isodynamic Law and the Law of Surface Area, and the determination of the calorie equivalents of foodstuffs along with other fundamental work, gave impetus to studies of energy metabolism.

Max Rubner was born in Munich on June 2, 1854. He received his early training under Carl Voit in Munich. During this time he was associated also with Pettenkofer. Voit and Pettenkofer had recently developed a method of estimating carbon dioxide, and this procedure enabled Rubner to carry out his studies. He also spent a year with Carl Ludwig at Leipzig.

When Rubner was twenty-four he embarked on experi-

ments to determine the rates of catabolism of carbohydrates, fats and proteins in fasting animals. One of his early contributions was the demonstration that 25 per cent of the total heat value of protein is lost to the body by the incomplete oxidation of nitrogen products. Following other studies Rubner came to the conclusion that the three major foodstuffs, carbohydrates, protein and fat could replace each other in accordance with their heat-producing value. This concept was termed the Isodynamic Law. At the time, he determined that 1 gm. of dry protein would produce 4.1 calories, 1 gm. of carbohydrate 4.1 calories, and 1 gm. of fat 9.3 calories during metabolism. These values are used, of course, universally. Voit was not in complete agreement with the Isodynamic Law, and full publication did not occur until 1883.

While still in Munich, Rubner also developed his Law of Surface Area. Following experiments on different species he concluded that in mammals, heat production was proportioned to the surface area. Using Meck's formula for calculating surface area he was able to show that the metabolism of a man per surface area was the same whether he was fasting or consuming a moderate diet. A similar relation was also found in animals and domestic farm birds.

In 1885 Rubner became Professor of Hygiene at Marburg. By 1889, he had with great effort and limited funds assembled and perfected a calorimeter. He carried out an experiment wherein a dog lived in the calorimeter for forty-five days. During this time 17,406 calories were calculated from expired carbon dioxide and excreted nitrogen. The calories measured by the calorimeter were 17,349, a remarkable agreement with those calculated. This elegant experiment established the law of conservation of energy in metabolism. No energy was available, except that resulting from chemical reactions.

Rubner was appointed Professor of Hygiene at Berlin in 1891. Eighteen years later he became Professor of Physiology, a position he held until his retirement in 1924. His work continued in Berlin. The surface area law was found to hold in a variety of species. Of particular interest to him was the "specific dynamic action" of foodstuffs, especially protein. He believed that specific dynamic action was due to the heat production of intermediary metabolism. Studies were conducted on the energy requirements during growth. Karl Thomas, working in his laboratory, showed that a man would excrete 2.2 gm. of nitrogen in the urine while on a starch and cream diet. Rubner termed this the "wear and tear" breakdown of protein, the minimum that would be excreted in the course of a protein-free input. In World War I, experiments were conducted on the nutritive values of different breads. It was found that white and rye bread were as nutritious as any other type.

Atwater and Lusk worked in Voit's laboratory while Rubner was there. Both were greatly influenced by Rubner and brought his ideas and methods to America. Benedict, in turn, worked with Atwater and then conducted his now classical studies of the fasting man in the Carnegie Institute in Washington. Lusk became Professor of Physiology at Cornell, where DuBois applied the methods to clinical research in the Russell Sage Institute at Bellevue Hospital. Benedict developed the current method of determining basal metabolic rates, and DuBois extensively studied caloric expenditure in a variety of disease states.

Thus the concepts of Rubner were seeded and grew in this country.

Lusk knew Rubner intimately and wrote of his friendship with him. He stated that, when Rubner was a young man, he liked to wear gay clothes and climb the Tyrolean mountains. However, in later life the dignity of the professional position demanded austere behavior and formality. Rubner was tall and of striking presence. He was often ruthless in debates with his colleagues. He liked to paint, and enjoyed relaxation among artist friends. Lusk quoted Karl Thomas as saying that Rubner was slow to give his confidence to anyone, but once he had given it he never withdrew it.

Rubner came to this country in 1912 to attend the International Congress of Hygiene and Demography in Washington. He visited Lusk in Long Island and journeyed to the Pacific Coast. He told Lusk that the Pacific Coast was finer than Nice, where he spent his Easter holidays.

In a later letter to Lusk, he stated that, though America was considered a "land of the dollar" in Europe, he found that young American scientists were dedicated despite their penurious salaries.

In the latter part of his retirement, Rubner became quite concerned about the welfare of the teaching profession. He wrote to Lusk in 1930 that something should be done about improving the retirement pay of teachers, as well as their salaries.

Rubner died in Berlin on April 27, 1932, a month before his seventy-eighth birthday. Shortly thereafter, Lusk delivered an address to a general session of the

American Association of the Advancement of Science. In this address he succintly described Rubner as having ". . . the power of arriving at great results through simple means, and of drawing correct conclusions from a few well-conducted experiments." Lusk considered Rubner the greatest man he had ever known.

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CORRESPONDENCE

To the Editor:

In an article which appeared in the November-December 1956 issue of DIABETES, H. S. Seltzer et al. state that glucose tolerance curves in which initial hyperglycemia is followed by a fall of blood sugar to hypoglycemic levels are diagnostic of mild diabetes mellitus, and that the hypoglycemia recorded in such curves constitutes an early manifestation of the diabetic state. Such interpretation of initially high glucose tolerance curves seems to me unjustified and fraught with the danger of making the diagnosis of diabetes mellitus in a number of nondiabetic subjects who may exhibit this type of blood sugar response to oral glucose loading.

The significance of glucose tolerance curves with an early excessive elevation of blood glucose and a secondary hypoglycemia has been a matter of concern for a number of years and, for the sake of proper historical perspective, it should be pointed out that such curves were first reported by Seale Harris in 1924.[1] Although he was aware that the curves may appear diabetic during the first three or four hours of the test he considered patients with this type of tolerance curve as potential, but not real, diabetics.[1, 2] A similar view was recently voiced by Skillern and Rynearson[3] who regard such curves as indicative of latent diabetes, while a position similar to that of Seltzer et al. was taken about a decade ago by Zondek et al.[4] who expressed the belief that such curves represent "joint occurrence of hyper- and hypoinsulinism."

In referring to my observations on glucose tolerance curves with initial hyperglycemia and secondary hypoglycemia[5, 6] Seltzer et al. make an entirely unfounded statement in saying that I have overlooked the existence of diabetes in such cases and have applied to them an erroneous diagnosis of functional hyperinsulinism. The fact, however, is that the diagnosis of diabetes in these cases was not overlooked, but simply ruled out for a number of reasons that were considered in great detail. Thus in my paper on "Clinical versus Laboratory Hypoglycemia"[5] in which thirty-three such curves were analyzed, I have brought out that: a. in seven curves the two-hour blood glucose readings were only slightly higher than normal; b. in nine the blood glucose values fell at first to 100 mg. per cent or less, and then rose to reach higher levels (so-called rebound curves); c. in none of the thirty-three tests did the peak glucose concentration in the capillary blood exceed the normal values; and d. in all thirty-three a fall to subnormal levels occurred in the second phase of the test. I have therefore concluded that "These findings do not support the diagnosis of diabetes, a condition in which the disorder of carbohydrate metabolism is reflected in a high, prolonged tolerance curve.

It is well known that hyperglycemia confined to the first two or three hours after administration of the loading dose may occur in various conditions unrelated to diabetes, such as the dumping syndrome, peptic ulcer, liver disease, hyperthyroidism, et cetera. For this reason, in absence of clinical manifestations of diabetes, an initially high glucose tolerance curve is not necessarily diagnostic of diabetes. A scrupulous search for the various conditions which may give rise to initial hyperglycemia in glucose loading tests was made in my material, but from the data presented by Seltzer et al., it appears that in their series only routine liver function tests were carried out with regard to the differential diagnosis of diabetes.

Another important point in the interpretation of initially high and secondarily low glucose tolerance curves is the fact that the curve pattern may vary in the same subject from one test to another. Seale Harris[2] stated: "one glucose tolerance test is not always sufficient to warrant a positive diagnosis of hyperinsulinism since there seem to be periods when the hyperinsulinism patient will have normal blood sugar readings, even though at other times he has shown marked hypoglycemia." My observations, on the other hand, show that the initial hyperglycemia of glucose tolerance tests

may also be replaced by a normal elevation of the blood glucose in a repeat tolerance test taken under identical dietary conditions. Lastly, Smelo[7] reported that in repeat tests carried out after a preparatory high carbohydrate diet there is a shift of the initial hyperglycemia toward or to normal values. It is therefore clear that the failure of Seltzer et al. to obtain repeat tolerance tests precludes any definitive statement concerning the diagnosis of diabetes in their patients.

In his early observations on hyperinsulinism, Harris suggested that prolonged excessive work of pancreatic islands may result in a deficiency of insulin secretion, that is, in diabetes.[1] The literature, however, records only one case[8] in which a patient with an initially high and secondarily hypoglycemic glucose tolerance curve developed diabetes within a comparatively short period of time (six months). According to Seltzer et al. several of their patients progressed to the stage of obvious diabetes, but long-range observations of Smelo[7] indicate that the development of overt diabetes in patients with such curves is roughly what it is in the general population. Clearly, it is highly unlikely that all patients reported by Seltzer et al. will one day become true diabetics.

As for the contention of Seltzer et al. that I have applied to my patients with an initially high glucose tolerance curve the diagnosis of functional hyperinsulinism, a glance at table 2 of my paper[5] will show that only four out of thirty-three such curves were recorded under the heading of spontaneous hypoglycemia and the remainder were diagnosed in relation to the clinical conditions which may be accompanied by spontaneous hypoglycemia or mimic the hypoglycemic syndrome.

The correction of statements made by Seltzer et al. about my observations is, of course, less important than the central point on which their own paper is based, that is, that their patients had at the same time both spontaneous hypoglycemia and diabetes. For reasons discussed above, I hold the view that they failed to establish the existence of diabetes in their patients, and I should like to present a strong plea against making the diagnosis of diabetes, a condition which stays with a patient for the rest of his life, exclusively on the strength of a transient hyperglycemia in but a single glucose tolerance test.

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To the Editor:

CONCERNING TRAUMA AND DIABETES

The more I think of the association of trauma with diabetes, the more I am concerned with it and, like Oliver Wendell Holmes, feel that "I am too much in earnest for either humility or vanity."

So far as I am aware, there is general agreement that trauma causes diabetes only when the pancreas is severely injured. The number of such cases which are accepted as reliable do not exceed a half dozen. However, the possibility has arisen that a latent diabetes might be accelerated and its appearance made manifest following an injury. No such instance has come to my attention among the 49,000 patients under our care since 1897, with sugar in the urine, of whom approximately 85 per cent are proved diabetics. I recall no such instance developing among their relatives, although constantly my colleagues and I are seeking to demonstrate an increased heredity in our patients, because we know at the first visit only about 20 per cent show a positive heredity, but after fifteen or twenty years of the disease this may be doubled and in the thirty-year duration cases of diabetes, trebled. Moreover, on our history sheets in two places we have the word "Trauma" printed, with the rule that a positive or negative finding should be recorded. I might also state we have many cases of trauma in connection with injuries to known diabetics and, because legal inplications frequently arise we take unusual pains to record details for use in court.

Believing all diabetes is hereditary, it can be accepted that latent diabetes occurs only in an individual who is hereditarily predisposed to it and has a diabetic gene even if the heredity is not proved. Overweight, pregnancy, an infection, involvement of thyroid, pituitary, adrenal glands or the liver are often precursors of dia-

betes. As time has gone on, fewer and fewer cases have been reported in which a doctor has thought an accident was followed by diabetes. Years ago, in order to be fair-minded to those who might differ with my own conviction that accidents do not cause diabetes, I stated the opposite side of the question in such a liberal fashion that it almost invited anyone to take it. Despite this it has not led to the publication of such instances.

In approaching the problem, I think one should remember that about 40,000,000 persons, one-fourth of the inhabitants of the United States, have a relative with diabetes and are thus hereditarily predisposed to it; that accidents are increasingly common and increasingly investigated; that no increase of diabetes was noted in Germany, France, England or the United States following and during the World Wars, either in the Armed Forces or in the civilian population (I personally saw but two cases at Mesves and after I left but one more was reported among these 35,000 soldiers who were within one day of the firing line); that no known diabetes has occurred following an injury in Harvard athletics; that Dr. Harvey Cushing, Dr. Gilbert Horrax and Dr. James L. Poppen have noted no case following operations upon the brain; that Dr. Donald Munro has seen no such case in accidents to the head coming under his observation. Indeed, so far as neurogenic diabetes is concerned, von Noorden said this was buried as a result of the first World War. It is not reported in suits for divorce and I have heard of no instance of a criminal awaiting execution who developed diabetes. Does a surgeon, doctor or a dentist put off an operation with the thought diabetes might appear?

Jacobsen demonstrated that trauma frequently led to glycosuria and increased sugar in the blood, but in his 140 cases this was always *temporary*. Therefore, a latent diabetic would not become a permanent diabetic because of trauma because diabetes would always revert to its former latency.

The mere fact that an individual has shown a trace of sugar in the urine one or more times in the past by no means proves it was due to diabetes. Probably if all specimens of urine of an individual were examined for a week, at least one of these would show a positive test for sugar. Furthermore, one must never forget that if individuals live on a low carbohydrate diet and after a week are given a glucose tolerance test, glycosuria or hyperglycemia or both temporarily may result.

Case No. 13,332, a 14-year-old Jewish boy, slept all night Christmas Eve in 1934 but the following night rose six times to void urine. When I saw him seventeen days later the urine contained 8 per cent sugar. No trauma, physical or emotional, occurred on Christmas Day.

I am writing this letter because I may have been overconsiderate in stating the opposite side of the question, although inspection of the context and conditions published in my original papers would make my position clear that an accident does not cause diabetes; second, my experience with diabetics is so great that I feel it my duty to report it; and finally, because of the handicap which would attach to one-fourth of the population of the country and all known diabetics and their relatives if it were considered likely that they would become diabetic if they underwent an injury.

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BOOK REVIEWS

DIABETES MELLITUS: HANDBOOK FOR PHYSICIANS. *By Howard F. Root, M.D., and Priscilla White, M.D. $7.00, pp. 346, Landsberger Medical Books, Inc., distributed solely by the Blakiston Division of the McGraw-Hill Book Co., New York, 1956.*

This new publication admirably achieves its objective in being an excellent book on diabetes with a detailed outline of the treatment both of the disease and the complications arising therein. It is clearly and concisely written so that "he who runs may read."

While the section on diet and diet therapy may be somewhat long the summary of the different types of insulin, their duration and mode of action is excellent.

The section dealing with acidosis and its management is clear and comprehensive.

Correctly the authors have devoted considerable time, thought and space to the management of complications of neuropathy, eye and skin. The results of their wide and varied experience are summarized and an evaluation made of the current best methods of management and treatment.

Since it has been estimated that one of three diabetics becomes a surgical patient sometime in the course of his disease, the surgical management of the diabetic is timely and well conceived.

The chapter on pregnancy in the diabetic is extensive and although there is considerable difference of opinion among experts now as to the value of hormone therapy in the pregnant diabetic, certainly Dr. White's statistics are very convincing.

The pages devoted to children born to diabetic moth-

ers will answer a great many questions asked by expectant diabetic mothers and their husbands. I know of no one who has had greater experience with these children or has studied them as carefully.

This is good reading for the diabetic specialist as well as for the man in general practice.

HYPOTHALAMIC-HYPOPHYSIAL INTERRELATIONSHIPS, *A Symposium—Third Annual Scientific Meeting of the Houston Neurological Society, Texas Medical Center. Chairman: Hebbel E. Hoff. Compiled and edited by William S. Fields, Roger Guillemin and Charles A. Carton, all of Baylor University. $4.75, pp. 176, illustrations 79, Charles C. Thomas, Springfield, Illinois.*

This volume is a series of eight papers that were presented as a symposium. In addition there is some discussion of each paper, and a general discussion forms the ninth chapter.

The papers were presented by men who were thoroughly acquainted with the problems and capable of presenting the evidence clearly and concisely. The result is a highly informative volume that reads easily. Most of the papers contain a great deal of data and they demand and merit careful study. A few comments on certain parts of the book will be made.

Neural Pathways to the Hypophysis, J. D. Green.

This is a good introductory chapter. It outlines the whole subject by giving a comprehensive and critical review of the literature and contains many stimulating comments.

Neurosecretion in the Central Nervous System, Walter Hild.

Dr. Hild presents the evidence that indicates that the Gomori staining granules in the neurohypophysis are the antidiuretic hormone or its precursor, that it is formed in the hypothalamus and migrates down the axons. He presents his own observations and discusses them in terms of the work of others. The argument is convincing.

Hypothalamic Control of the Anterior Lobe of the Hypophysis, G. W. Harris.

The author commences by stating that the hypothalamus regulates the anterior pituitary. He then reviews the evidence demonstrating the importance of the pituitary portal system. He presents both sides of the question and gives the technical difficulties which may cause erroneous results. He is able to assess these difficulties from his own laboratory experience. His conclusions are well supported by the evidence presented.

Telencephalic Influences upon the Hypothalamus, Pierre Gloor.

In this chapter, which represents almost one quarter of the book, there is an excellent outline of the anatomy and functional relations between the limbic system, the hypothalamus and the midbrain. Gloor begins with an adequate outline of the anatomical pathways concerned, then proceeds to show some of the physiological relations between these various structures, using the method of stimulation and electronic recording. A review of ablation experiments provides complementary evidence of the functions and emphasizes the importance of the amygdaloid projection system.

Gloor reviews the evidence and is able to propose a process which places the limbic system as a coordinator of neocortical and midbrain function while providing a mechanism for homeostatic and emotional activity.

HORMONES AND THE AGING PROCESS. *Proceedings of a Conference held at Arden House, Harriman, New York, 1955. Edited by Earl T. Engle and Gregory Pincus. $8.50, pp. 325, illus., Academic Press, New York, 1956.*

Immortality is no nearer despite the concentrated effort of the distinguished panel of endocrinologists participating in a conference on aging held at Arden House. The conference report includes eighteen papers presenting a massive amount of data on measurable changes in endocrine function with advancing age and in certain disease states. The majority deal with the steroid hormones, particularly the androgens and estrogens and their influence upon anabolism.

White, in a concise review, summarizes the evidence showing that insulin is essential in the synthesis of body protein and can facilitate this process even in the absence of growth hormone. He ascribes this action of insulin to its influence upon carbohydrate metabolism.

The treatment of osteoporosis with steroid hormones and Vitamin D is described, and studies in treatment of breast cancer are reported. Reference is made to hypophysectomy and adrenalectomy as well as to the administration of estrogens and androgens.

With few exceptions, the papers are authoritative and critical. Some are controversial. Most are carefully scrutinized by the panel in the discussion which follows each paper, affording the reader the reaction of trained investigators to work that would otherwise be difficult to evaluate. The comments are lively, constructive and, occasionally, sharp.

ABSTRACTS

Andrews, C. T. (West Cornwall Clinical Area, England): A SURVEY OF DIABETES IN WEST CORNWALL. Brit. M. J. *1*:427-33, Feb. 23, 1957.

The author reports the results of the diabetes survey in West Cornwall, where the incidence of the disease was found to be 5.6 per cent per 1,000 population. This figure is high compared with previously published figures for the country as a whole. The sex distribution (three female: two male) follows that of the country as a whole. A study of the age of onset showed a tendency for male predominance up to the age of forty, with a striking excess of females in the sixth and seventh decades and a tendency for male predominance to reappear in old age. Less than 50 per cent of the patients presented classical symptoms. Seventy-seven per cent of the patients attended a medical or diabetic clinic. The problems of the home were mainly those of insulin administration and intercurrent illness: 80 per cent of the male and 62 per cent of the female diabetics requiring insulin administered it themselves. More than three-fourths of the patients attending the clinics had no illness requiring bed rest during the course of a year. The female patients tended to have more illness than the male. Respiratory illness predominated in the home. Diaebtes and its complications were responsible for only 15.5 per cent and 13.8 per cent of all days in bed in the two years investigated. The district nurse visited 15.1 per cent of all diabetic patients during a year and was responsible for daily insulin administration in about 6.4 per cent of all patients. The general practitioner saw the average diabetic patient ten times during the year. He was consulted by 91 per cent at some time during the year. The commonest cause for hospital admission was initial stabilization: 42.5 per cent of all admissions were for reasons in connection with the diabetes. The author suggests that one medical bed for every 100 diabetics is required in this area. Cardiovascular disease was responsible for death in more than half of seventy-nine patients followed up. The disease in Cornwall appears to affect life expectancy in the female but not in the male. The age of death for both sexes is 67.1 years.

Annotation: DIABETES MELLITUS AND ANAESTHESIA. Lancet *1*:465, March 2, 1957.

Preparation of diabetic patients for general anesthesia is commented on. The author's views are stated.

Bastenie, P. A.; Franckson, J. R. M.; de Meutter, R.; Demanet, T. C.; and Conrad, V. (Univ. Dept. of Med., St. Pierre Hosp., Brussels, Belgium): METABOLIC EFFECTS OF CARBUTAMIDE IN SELECTED DIABETICS. Lancet *1*:504-07, March 9, 1957.

The authors report their experience with eighteen diabetics given carbutamide therapy. The results of such therapy and implications drawn from these data are presented.

Bloom, Roy A.; and Fenton, Paul F. (Dept. of Biol., Brown Univ., Providence, R. I.): GLUCOSE TOLERANCE IN RELATION TO OBESITY AND FOOD INTAKE. Am. J. Physiol. *184*:438-40, February 1956.

Glucose tolerance tests were carried out on two strains of mice, one highly susceptible, the other only moderately susceptible to nutritional obesity. With administration on the basis of body weight or fat-free weight, the highly susceptible strain disposed more rapidly of the administered glucose than did the moderately susceptible strain. Fasting blood sugar levels in both strains were found to increase with the logarithm of body weight. These observations are not readily explained in terms of the "glucostatic" theory of the control of food intake, which holds that elevated blood sugar levels suppress hunger and food consumption.

Burge, Dan (Dept. of Med., Emory Univ. Sch. of Med., Atlanta, Ga.): INSULIN EDEMA. J. M. A. Georgia *45*:428-30, October 1956.

A case is presented in which a newly diagnosed diabetic exhibited severe fluid retention over a period of five months and to some degree for nearly two years, whenever insulin was taken. Various possible modes of pathogenesis have been discussed.

Burt, Richard L. (Dept. of Obst. and Gynec. of the Bowman Gray Sch. of Med. of Wake Forrest Coll. and North Carolina Baptist Hosp., Winston-Salem, N. C.): PERIPHERAL UTILIZATION OF GLUCOSE IN PREGNANCY. Obst. & Gynec. *9*:310-16, March 1957.

Insulin sensitivity in pre-eclampsia is significantly less than the physiologic loss of insulin reactivity, developing in late normal pregnancy. This is characterized by a lessened hypoglycemia as well as by "lag" in the response of the plasma in organic phosphorus following insulin administration.

It is suggested that corticosteroid influences may be responsible for the changes noted.

Cavert, H. Mead; and Johnson, John A. (Dept. of Physiol., Med. Sch., Univ. of Minnesota, Minneapolis, Minn.): METABOLISM OF CARBOXYL-LABELED SHORT CHAIN FATTY ACIDS BY THE ISOLATED DOG HEART. Am. J. Physiol. *184*:582-88, March 1956.

A comparative study has been made of the metabolism of 2-, 3-, 4-, and 8-carbon carboxyl-labeled fatty acids to CO_2 and to glycogen in the isolated dog heart perfused with heparinized blood by the Langendorff technic. In confirmation of previous work by others, this compound contributed 30 to 40 per cent of respiratory CO_2 at high concentrations of administered acetate-1-C^{13}. Increasing the level of administered acetate correspondingly elevates the contribution of acetate carbon to CO_2. The portion of total CO_2 derived from 2-, 3-, 4-, and 8-carbon fatty acids appeared comparable (5 per cent) at substrate levels of 1-2mM/L. However, at 12-14 mM/L the values were 20, 5, 3, and 3 per cent of total CO_2 derived from the C^{13}-labeled carboxyl carbon of acetate, propionate, n-butyrate, and n-octanoate respectively. On the assumption of equal rates of combustion of all carbon atoms in each compound, the percentages were 41, 15, 13, and 25 per cent, respectively, of CO_2 derived from the administered labeled substrate. Labeled sodium formate was not rapidly converted to respiratory CO_2 in a single isolated heart experiment. No carboxyl carbon from any of the labeled fatty acids contributed detectably to carbon of cardiac glycogen.

Clayton, S. G. (King's College Hosp. and Queen Charlotte's and Chelsea Hosps., London, England): THE PREGNANT DIABETIC: A REPORT ON 200 CASES. J. Obst. & Gynaec. Brit. Emp. *63*:532-41, August 1956.

This report is based on the records of 201 diabetic pregnancies (in 176 patients) seen at King's College Hospital since 1949. There was only one maternal death, although 122 cases were delivered by cesarean section and six by abdominal hysterotomy and labor was induced in most of the others. In spite of this over-all mortality of 0.5 per cent, it would be wrong to imply that little maternal risk is involved in these cases. Even with careful supervision, three cases were reported as being in a state of "precoma" on some occasion, and severe ketosis was noted in several others. The difficulties of control are partly due to the lowering of the renal threshold for glucose occurring so often in pregnancy. This does render not only the ordinary control by urinary testing unreliable, but it may lead to ketosis because of the wastage of sugar. In most cases (though not in all) the insulin requirement rises during pregnancy, although the dose may need to be reduced again in late pregnancy; and it must not be forgotten that it often falls abruptly in the puerperium. In spite of careful control of diabetes, the fetal mortality remains high, being 28 per cent among cases delivered after the twenty-eighth week. The fetal prognosis is very bad in cases where albuminuria is found in early pregnancy and also in cases of essential hypertension; but, on the other hand, late pre-eclamptic toxemia is not a common cause of intra-uterine fetal death. There is a possibility that many of these intra-uterine deaths are due to anoxia. In this series thirty neonatal deaths did occur. Most cases followed caesarean section, but only, probably, as this has been the commonest method of delivery. The child is delivered together with the usual flood of liquor. Its immediate condition is excellent, and it usually cries at once. Often large quantities of fluid are aspirated from the air passages, and gastric aspiration yields unusual quantities of fluid. All remains well for a time; but after a few hours respiratory distress begins, with successive cyanotic attacks, each of which becomes more severe until circulatory failure occurs. This mode of death was seen in twenty-three out of thirty cases. Absorption atelectasis was found, owing to hyaline membrane formation in the air passages in every case where an autopsy was performed. The average time from birth to death was thirteen hours, the shortest, three hours. All but one of the deaths occurred within twenty-four hours. Five neonatal deaths were due to severe congenital abnormalities. The pattern of these varied greatly. It is not certain that the incidence is higher in diabetic than in nondiabetic cases. The two remaining neonatal deaths were due to intracranial injury during vaginal delivery, and the large size of the infants should be a factor increasing the risk of this accident.

Colwell, Arthur R. (Northwestern Univ. Med. Sch. and Passavant Memorial Hosp., Chicago, Ill.): TREATMENT OF DIABETIC ACIDOSIS. A. M. A. Arch. Int. Med. *99*:260-65, February 1957.

The chief elements in skillful treatment of diabetic acidosis are: (1) prevention by eliminating its common causes in advance; (2) accurate diagnosis; (3) correct appraisal of severity; (4) thoughtful and discriminating use of crystalline insulin, with replacement of depleted water and salts, and of sugar later, when needed.

Croom, J. Halliday (Physician, Royal Infirmary, Edinburgh, Scotland): RENAL ASPECTS OF DIABETES. The Medical Press *236*:589-91, Dec. 19, 1956.

It has been well known for many years that diabetics who become ketotic and go into diabetic coma are liable to show albuminuria associated with glycogen and fat

deposits in the kidney tubules. Infective lesions of the kidney have also been known for a long time to be more common in diabetics than in nondiabetics. It was believed at first that "diabetic nephropathy," the term commonly used to describe degenerative renal disease in the diabetic, was largely confined to the older age group. With the years, however, it has become apparent that it occurs quite commonly in young people, and is directly related to two factors, namely, the duration and the control of the diabetic state. The severity of the diabetes, as shown by the amount of insulin required, does not appear to bear any relation to the production of kidney lesions. It must be emphasised, however, that the presence of the lesion in the kidney is not necessarily associated with symptoms or physical signs. Once degenerative complications have been established, there is really little one can do except to try and keep the patient as comfortable as possible. Salt restriction is probably worth while, once edema becomes troublesome.

Croom, J. H.; Duncan, L. J. P.; Dunlop, D. M.; Macgregor, A. G.; and Meiklejohn, A. P. (Royal Infirmary, Edinburgh, Scotland): INSULIN ZINC SUSPENSIONS. Lancet *2*:1216-17, Dec. 8, 1956.

The authors report on their experience with insulin zinc suspensions in regulating the insulin dosage of a large series of diabetic patients.

Davies, Mary H.; and Paley, Ronald G. (Dept. of Med., Univ. of Leeds, Leeds, England): A QUICK AND EASY METHOD FOR BLOOD-SUGAR ESTIMATION. Brit. M. J. 1:501-02, March 2, 1957.

The authors report a new, simple, and quick method for blood sugar estimation. Results are in remarkable agreement with those obtained with the Hagedorn-Jensen method. Precautions and pitfalls regarding this test are described. The new blood sugar method would appear to be helpful in medical emergencies.

Drury, Douglas R.; and Wick, Arne N. (Dept. of Physiol., Sch. of Med., Univ. of Southern California, Los Angeles, and the Scripps Metabolic Clin., La Jolla, Calif.): METABOLISM OF LACTIC ACID IN THE INTACT RABBIT. Am. J. Physiol. *184*:304-08, February 1956.

The metabolism of lactic acid was studied in normal intact rabbits with the use of C^{14}-labeled L (+) lactate. The circulating lactate is being used up and renewed at a rapid rate. The turnover time is about thirty minutes. Eighty to 90 per cent of the lactate is disposed of by direct oxidation. Only a small percentage can be accounted for as glucose or glycogen or by the oxidation of these. The discrepancy between these results and those previously reported by other workers, who found large increases in liver glycogen and little oxidation of lactate, may be attributed to the fact that the natural isomer was employed in the present experiments and racemic lactate in earlier investigations. Lactate, produced in emergency states, can serve as a quick fuel for vital tissues in these situations.

Drury, M. I. (Natl. Maternity Hosp., Dublin, Ireland): INSULIN ZINC SUSPENSIONS. Lancet *2*:1358, Dec. 29, 1956.

The author comments on the use of insulin zinc suspensions, with special reference to pregnant diabetics.

Editorial: DIABETICS AND DRIVING (Annotation). Lancet *1*:310, Feb. 9, 1957.

The author discusses the problems encountered by diabetics driving motor vehicles and cites recent pertinent literature on this subject.

Editorial (*Medico-Legal Correspondent*): INSULIN AND DRIVING. Brit. M. J. *1*:349, Feb. 9, 1957.

The author discusses the problems involved when diabetics taking insulin drive motor vehicles. The judicial aspects of such a situation are also discussed.

Editorial: TOLBUTAMIDE (Annotation). Lancet *1*:259-60, Feb. 2, 1957.

The author comments on the sulfonylurea drugs, currently under investigation as hypoglycemic agents. Special reference is made to tolbutamide as the one still being investigated. With reference to this drug his outlook is both cautious and pessimistic.

Egdahl, Richard H.; and Richards, John B. (Naval Med. Res. Inst., Natl. Naval Med. Center, Bethesda, Md.): EFFECT OF CHLORPROMAZINE ON PITUITARY ACTH SECRETION IN THE DOG. Am. J. Physiol. *185*:235-38, May 1956.

Effect of chlorpromazine on adrenal cortical function has been determined by measuring 17-hydroxycorticosteroids in adrenal venous blood of dogs, following intravenous injection of the drug. It was found that approximately half of the animals had significant adrenal responses to the injection of 1.3-3.3 mg./kg. of chlorpromazine and that all animals responded to dosages of 3.5 mg./kg. and above. The adrenal response was abolished by hypophysectomy, which ruled out a direct action of the drug on the adrenal gland. The response was unaffected by the maintenance of high peripheral blood levels of hydrocortisone. These observations suggest that chlorpromazine stimulates either directly or through the hypothalamus the release of ACTH from the adenohypophysis. The observation that frequently the steroid output from the adrenal does

not reach its maximum until six to twelve minutes after the chlorpromazine is injected is in favor of the latter view.

Eik-Nes, Kristen; and Brizzee, Kenneth R. (Dept. of Biol. Chem. and Anat., Univ. of Utah College of Med., Salt Lake City, Utah): ADRENOCORTICAL ACTIVITY AND METABOLISM OF 17-HYDROXYCORTICOSTEROIDS IN THYROIDECTOMIZED DOGS. Am. J. Physiol. *184*:371-75, February 1956.

Low or zero levels of plasma 17-hydroxycorticosteroids and a reduced adrenocortical capacity were found in dogs at the seventh day following thyroidectomy. The daily administration of l-thyroxine to thyroidectomized dogs restored normal adrenocortical function. Low but measurable levels of plasma 17-hydroxycorticosteroids were present in dogs thirty days after thyroidectomy, whereas an apparent normal removal rate of intravenously given cortisol (compound F) was present both at the seventh and at the thirtieth day after thyroidectomy. Since sham operations or other "stress" forms were not associated with reduced adrenocortical function either on the seventh or on the thirtieth day post-"stress," a thyroid-adrenal metabolic interaction is postulated, with a predominant production of thyrotropic hormone and a reduced production of ACTH after thyroidectomy.

Friedman, Meyer; Bine, René; and Ishida, Tad (Harold Brunn Inst., Mt. Zion Hosp., San Francisco, Calif.): REMOVAL OF EXCESS CHOLESTEROL AND CHOLATE BY THE ISOLATED RAT LIVER FROM ITS PERFUSATE. Am. J. Physiol. *184*:412-14, February 1956.

Perfusion of the isolated rat liver with a solution containing hypercholesteremic and hypercholatemic blood results in the removal of some of the cholesterol and almost all of the excess cholate. The withdrawn cholesterol is deposited almost completely in the liver, whereas the withdrawn cholate is excreted promptly in the bile. It is concluded that the isolated liver behaves in a manner qualitatively similar to that of the liver of the intact rat in respect to cholesterol and cholate metabolism.

Gassmann, W.; and Schneeweis, J. (Städt. Krankenhaus, Berlin-Hohengatow, Germany): DIABETES MELLITUS AND PERNICIOUS ANEMIA. Acta. Haemat. *16*:396-99, December 1956.

The incidence of pernicious anemia in 1,797 diabetics was compared with that of 6,313 nondiabetics. The association of pernicious anemia with diabetes was significant as compared with the nondiabetic control group. The authors suggest that vitamin deficiencies may be the cause of the increased coincidence of both diseases.

Gibbs, Gordon E.; and Henn, Mary J. (Depts. of Pediat. and Internal Med., Univ. of Nebraska Coll. of Med., Omaha, Nebr.): THE ADRENAL CORTEX IN RELATION TO RETINAL AND RENAL VASCULAR LESIONS IN DIABETES. Nebraska M. J. *41*:427-29, November 1956.

Present evidence suggests that incomplete diabetic control leads to an adrenocortical hyperactivity, which in turn contributes to development of glomerulosclerosis and retinitis. Encouraging but as yet only experimental results have been obtained in a few cases, in attempts to interrupt this sequence of events by adrenalectomy or hypophysectomy. The most practical approach to the problem of degenerative vascular changes would still appear to be preventive, namely, more complete utilization of presently available means of control of the diabetes by insulin and diet.

Gifford, H. (Omaha, Nebr.): OCULAR MOTOR NERVE PARESIS IN DIABETES MELLITUS: CASE REPORT. Nebraska M. J. *41*:429-30, November 1956.

The exact etiology in this type of case has always been doubtful. For the most part, it has been considered to be due to a vascular accident; but in the last reported cases, it appeared more to be of a neuritic condition than a vascular one. This conclusion was primarily due to the fact that these cases all recovered spontaneously. Reports in the literature show that the nerves most frequently affected are the third and sixth; but in this case, the fourth nerve was involved. Paralysis was not complete, but there was severe enough paresis as to produce annoying diplopia, which diminished promptly but not completely after two months.

Gillison, T. H. (Ilkeston, England): ENZYME TEST FOR GLYCOSURIA. Brit. M. J. *2*:1487-88, Dec. 22, 1956.

The author comments on the ease with which enzyme test for glycosuria is carried out.

Gitelson, S.; and Wislicki, L. (Rothschild-Hadassah Univ. Hosp. and Dept. of Pharmacol., Hebrew Univ., Hadassah Med. Sch., Jerusalem, Israel): USE OF ACTH IN THE TREATMENT OF INSULIN RESISTANT DIABETES: CASE REPORT. Harefuah. 50:270, June 15, 1956.

A fifty-five-year-old man had been diabetic for six years but required insulin only for the last six months. After the death of a sister in diabetic coma two months before admission, his insulin requirement rose gradually. Following admission, PZI was discontinued because of local reactions, and soluble insulin in three separate doses as well as Benadryl and Terramycin were used. Nevertheless, the blood sugar and glycosuria level remained high until, on the twenty-seventh day of hospitalization, the daily dose of insulin was finally raised to 520 units. ACTH gel, in doses of 20 to 40

units per day, was begun on the thirty-sixth day; and within twelve days, the daily dose of insulin could be reduced to 40 units.

Glicksman, Arvin S.; and Rawson, Rulon W. (Dept. of Med., Memorial Hosp. and Div. Clin. Investigation, Sloan-Kettering Inst., Memorial Center for Cancer and Allied Diseases, New York, N. Y.): DIABETES AND ALTERED CARBOHYDRATE METABOLISM IN PATIENTS WITH CANCER. Cancer *9*:1127-34, November-December 1956.

The observation of spontaneous hyperglycemia in patients with cancer and the presence of an increased incidence of diabetes in the cancer population is not a new one. Early workers in the field sought a readily available diagnostic tool for cancer; however, as it became apparent that the glucose tolerance curve was not altered in anywhere near 100 per cent of cancer patients, they lost their enthusiasm. Similarly, when it became evident that the presence or absence of altered carbohydrate metabolism could not be correlated with response to therapy, investigation in this field waned again. On the basis of the 950 cases reported in this survey, altered carbohydrate metabolism, as well as overt diabetes, appears to be significantly associated with neoplasia. The elucidation of the mechanism by which this occurs may yield insight into the basic problem of cancer.

Goodbody, R. A. (Southampton, England): LESIONS OF THE FEET IN DIABETES. Brit. M. J. *1*:340, Feb. 9, 1957.

The author refers to an article in the recent diabetic literature concerning this problem.

Gray, Doris E.; and Deluca, H. A. (Dept. of Biochem., Univ. of Western Ontario, London, Ont., Canada): USE OF DESOXYRIBONUCLEIC ACID AS A REFERENCE STANDARD IN METABOLIC EXPERIMENTS. Am. J. Physiol. *184*:301-03, February 1956.

The metabolic activity (as gauged by glycogen synthesis, glucose uptake, and oxygen consumption per gram of wet tissue) was found to be increased in diaphragms removed from rats which had been maintained on a low-protein diet as compared with normal controls. No significant difference appeared in these indices of metabolic activity when they were expressed on a per cell basis (calculated on the content of desoxyribonucleic acid phosphorus). In protein deprivation, the mass of the diaphragm cell was dependent upon the protein intake rather than upon the total caloric intake, but the metabolic activity of the cell was independent of its mass. These findings suggest that the metabolic activity of a tissue may, under certain experimental conditions, be more suitably expressed on a per cell basis than on the more usual basis of wet weight of tissue.

Helps, E. P. W. (Dept. of Med., Johns Hopkins Hosp., Baltimore, Md.): SENILE CONFUSION AND THE BLOOD-SUGAR LEVEL. Lancet *1*:138-39, Jan. 19, 1957.

The author discusses the occurrence in elderly patients of attacks, resembling hypoglycemia but characterized by a relatively high blood sugar. The case histories reported suggest that, in the elderly diabetic with arteriosclerosis and, consequently, reduced blood flow, cerebral metabolism may become adapted to a supply of glucose in higher concentration than normal and may be disturbed (in some cases irremediably) by reduction to normal of the blood sugar. The author suggests further that this type of patient may be managed more satisfactorily by a deliberate control aimed at persistent hyperglycemia.

Hewitt, John E.; and Hayes, Thomas L. (Donner Lab. of Med. Physics. Radiation Lab., Dept. of Physics, University of California, Berkeley, Calif.): X-IRRADIATION AND LIPOPROTEIN METABOLISM IN VARIOUS SPECIES. Am. J. Physiol. *185*:257-64, May 1956.

The authors present changes in lipoprotein concentration, following x-irradiation for the rabbit, dog, rat, and mouse. All of these species showed alterations in their lipoprotein concentrations during the post-irradiation period. A pronounced decrease in high-density lipoproteins occurs in the mouse. A relatively large increase in concentration of low-density lipoproteins precedes death from irradiation in the rabbit, rat, and dog.

Hollett, Charlotte; and Meng, H. C. (Dept. of Physiol., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): COMPARISON OF FAT EMULSION CLEARING ACTIVITIES OF POSTHEPARIN PLASMA AND PANCREATIC LIPASE. Am. J. Physiol. *184*:428-32, February 1956.

Work has been carried out comparing the properties of the lipemia-clearing factor of postheparin plasma and pancreatic lipase. It was found that both postheparin plasma and pancreatic lipase exhibited lipolytic activity and were capable of clearing the turbidity of neutral fat emulsion. Intravenous administration of pancreatic lipase was able to clear alimentary lipemia, and post-lipase plasma was also capable of clearing the turbidity of neutral fat emulsion in vitro. Study of the effects of various chemicals and agents upon the relative activities of postheparin plasma and lipase indicated that the lipemia-clearing factor of postheparin plasma was not identical with pancreatic lipase or serum lipase of pancreatic origin. No appreciable change in plasma lipase was observed, following intravenous administration of heparin. It is suggested that the lipemia-clearing factor of postheparin plasma might be a specific esterase which is capable of splitting the ester bonds of neutral fat of long-chain fatty acids.

Howard, Everett E.; and Marlette, Robert H. (Kansas City Genl. Hosp., Kansas City, Mo.): THE RATIONALE OF MANAGEMENT IN ORAL SURGERY PROCEDURES ON DIABETICS. Oral Surg. *9*:1032-39, October 1956.

The oral surgeon who contemplates surgery on a diabetic patient should be cognizant of the therapeutic problems confronting the internist in the medical management of the patient. The awareness of diabetic therapy by the dentist potentiates accurate treatment planning which conforms with the need and requirements of the diabetic patient who has to undergo surgery. Postoperative complications are effectively prevented or minimized by hospitalization and careful attention to individual "stress" reactions. It must be emphasized that the internist should carry out the management of diabetic patients undergoing surgery. Each patient requires individual management, and the success of surgery may depend upon the preoperative and postoperative care given by the internist.

Joiner, C. L.; and Lee, C. T. (Pennsylvania Hosp., Diabetic Clin., Philadelphia, Pa.): A CONTROLLED TRIAL OF CARBUTAMIDE IN DIABETIC PATIENTS: AN INTERIM REPORT. J. Michigan M. Soc. *55*:1199-1203, 1217, October 1956.

Two methods are described for evaluating the response of diabetic patients to the sulfonylureas, one suitable for use with hospitalized patients and the other for outpatients. Both methods permit control observations to be made. When controls are not used, the evaluation of patient's response to hypoglycemic agents may be difficult and misleading. Three patterns of response have been demonstrated by each method: unequivocal success, unequivocal failure, and progressive improvement which persists for as long as two weeks, following withdrawal of the drug. Not responding patients are unstable juvenile diabetics, requiring relatively large doses of insulin. The most dramatic improvement in control is observed in mild obese diabetics of the older age groups, who do not need insulin as a general rule. A final appraisal of the usefulness of the sulfonylureas in the treatment of diabetes mellitus cannot be made at the present time.

Kerlan, Martin (Mountain Home, Tenn.): TRIAL EXPERIENCES WITH THE ORAL ANTIDIABETIC AGENT BZ-55. J. Tennessee M. A. *50*:46-48, February 1957.

Thirteen patients were carefully studied over a six-month period, during which time it was considered necessary to stop the drug in three patients. It was observed that the mild diabetic responds well insofar as his blood sugar is concerned. The further factor of overenthusiasm on the part of the patients comprises a hazard in the decision as to the use and continuation of this drug. This enthusiasm far outweighs any observable benefits.

Kim, S. Kwang; Bollman, Jesse L.; and Grindlay, John H. (Sects. of Biochem. and of Surg. Res., Mayo Clin. and Mayo Foundation, Rochester, Minn.): METABOLISM OF ENDOGENOUS LIPID OF THE INTESTINE. Am. J. Physiol. *184*:445-48, March 1956.

Total fatty acid output in the intestinal lymph of rats and in the thoracic-duct lymph of dogs was studied during fasting and during a fat-free diet period. Daily total fatty acid output in the intestinal lymph was found to be fairly constant during fasting or when the rat was fed a fat-free diet. An inverse relationship exists between total fatty acid concentration and the volume of intestinal lymph. In the absence of pancreatic juice or of both bile and pancreatic juice, the total acid output in the thoracic-duct lymph of dogs was decreased to one-third or one-fourth of that of the normal dog. Endogenous fat in the intestinal lymph represents the reabsorbed portion of the endogenous lipid, and endogenous fecal fat represents the unabsorbed portion of endogenous lipid excreted into the gastrointestinal lumen. Fecal fat derives from two sources, exogenous dietary fat and endogenous lipid.

Kits van Heijningen, Adrienne J. M. (Pharmaco-therapeutic Lab., Univ. of Amsterdam, Amsterdam, Holland): THE INFLUENCE OF INSULIN ON FREE AND FIXED GLYCOGEN IN THE DIAPHRAGM OF THE RAT. Biochem. J. *65*:111-15, January 1957.

The author reports the effect of insulin on free and fixed glycogen of rat diaphragm in vitro. The increase in glycogen content, brought about by insulin, is limited to the free fraction. The significance of this finding and the possible roles of both glycogen fractions in muscular contraction are discussed.

Kohn, J. (Queen Mary's Hosp., Rochampton, London, S.W. 15, England): A RAPID BLOOD-SUGAR SCREENING METHOD. Lancet *1*:213, Jan. 26, 1957.

The author reports on the use of glucose enzyme test strips in determining blood sugar levels. A full report is in preparation.

Kracht, Joachim; Kröner, Barbara; v. Holt, Linde; and v. Holt, Clause (Physiologisch-Chemisches Institut und Pathologisches Institut der Universität, Hamburg, Germany): INCREASES OF PLASMA-INSULIN-ACTIVITY AND BETA-CELL MITOSES AFTER N-(4-METHYL-BENZOL-SULFANYL)-N-BUTYL-UREA. Naturwissenschaften *44*: 16-17, 1957.

The rate of beta-cell mitoses was determined through the colchicine test; male Sprague-Dawley rats were

treated with 500 mg./kg. of D-860 for twenty-one days. The animals were sacrificed twenty-four hours after the last medication and nine hours after subcutaneous injection of 0.15 mg. of colchicine. An average of seventy-six mitoses per 100 islets were counted in the pancreas of the treated animals, whereas untreated control animals showed only twelve mitoses per 100 islets.

The plasma insulin activity was determined according to Randle's hemidiaphragm technic. Plasma was obtained from fasted male Sprague-Dawley rats two hours after a single oral dose of 300 mg./kg. D-860. Twenty-four control animals and sixteen treated animals were used. Determinations were made in the mixed plasma of two animals each. The treated plasma had an insulin activity of 101.3 mEq./0.5 ml.; the control plasma showed an activity of only 14.6 mEq./0.5 ml. The more than sixfold increase in both plasma insulin activity and beta-cell mitoses after administration of D-860 are similar to the increases obtained by the same authors previously with BZ-55. The findings are presented as evidence that these hypoglycemic sulfonylurea compounds act essentially via a stimulation of the beta-cells and of insulin production and secretion.

Krieger, Ingeborg; Anderson, Walter L.; and Kelson, M. J. (Harper Hosp., Detroit, Mich.): DIABETES IN A ONE-YEAR-OLD INFANT. Harper Hosp. Bull. *14*:134-39, July-August 1956.

Presented is the case of an infant who at an age of twelve months developed diabetes with subsequent acidosis and coma. Discussed are the diagnostic difficulties of threatening diabetic coma encountered in infants. Factors which come into play in childhood and may precipitate the onset of the disease are mentioned, with special emphasis on infections. The prognosis of diabetes and diabetic coma in infants and children is discussed in its relation to the various methods of treatment used in the past.

Leonards, Jack R. (Dept. of Biochem., Sch. of Med., Western Reserve Univ., Cleveland, Ohio): EVALUATION OF ENZYME TESTS FOR URINARY GLUCOSE. J. A. M. A. *163*:260, Jan. 26, 1957.

Clinistix (Ames) and Tes-Tape (Urine Sugar Analysis Paper, Lilly), two commercial specific enzyme preparations for testing for urinary sugar, were found to be useful for the qualitative detection of urinary glucose. Tes-Tape was found to be satisfactory as a quantitative method for urines containing 0.25 per cent glucose or less. For urines containing more than 0.5 per cent glucose, the use of Tes-Tape as a quantitative procedure is unsatisfactory and can lead to serious error in the interpretation of a patient's clinical condition.

Loder, L. L. (Muskegon, Mich.): DIABETIC RETINOPATHY FOR THE GENERAL PRACTITIONER. J. Michigan M. Soc. *55*:1204-06, October 1956.

It is well agreed that more violent types of retinopathy are prone to occur when diabetes presents itself early in life. These are the cases where diabetes is more difficult to control, largely because of difficulty in patients' co-operation. The rapidity of breakdown has a fairly direct relationship to the control of diabetes, when retinopathy occurs in the adult. Prophylactic emphasis on meticulous control of the diabetes is still the best form of treatment rather than are attempts to rectify end results of deterioration. Along with the medical control of diabetes goes physical and psychologic control. In this connection, Duke-Elder lists these four points in order of importance: (1) dispel anxiety, (2) reduce speed, (3) lessen intake and promote elimination, and (4) exclude toxemia.

Luntz, George (Birmingham Chest Clin.; Birmingham Regional Hosp. Board, Birmingham, England): A SIMPLE QUICK TEST FOR GLUCOSE IN URINE: REPORT ON USE OF CLINISTIX. Brit. M. J. *1*:499-500, March 2, 1957.

The author reports a study showing the reliability of Clinistix in detecting urinary glucose.

McConahey, William M.: THE DEGENERATIVE COMPLICATIONS OF DIABETES. Minnesota Med. *39*:815, Dec. 1956.

No effective treatment is known as yet, once the vascular complications of diabetes are developing. At the present time, careful control of the diabetes is obviously the only measure which seems likely to delay or prevent the appearance of the complications, and this should be emphasized to all diabetic patients.

Magee, D. F.; and Hong, S. S. (Dept. of Pharmacol., Univ. of Washington Sch. of Med., Seattle, Wash.): CHANGES IN PANCREATIC ENZYMES BROUGHT ABOUT BY AMINO ACID ADDITIONS TO THE DIET. Am. J. Physiol. *184*:449-52, March 1956.

Rats fed 7 per cent casein as their sole source of protein were divided into eight groups. Two of these were kept as controls, receiving an extra 1 per cent of casein. The remaining groups were fed equal amounts of a diet containing 1 per cent of the following dl amino acids: isoleucine, methionine, phenylalanine, leucine, lysine, or tryptophane. At the end of 3 weeks' feeding, all the animals were killed after a twenty-four-hour fast and the enzyme activities in the pancreas determined. By

comparison with the controls, it was found that no amino acid supplement increased amylase. Methionine increased lipase and protease activity, and phenylalanine and isoleucine increased protease activity alone.

Mohnike, G.; and Worm, M. (Diabetikerheim, Karlsburg, Germany): THE RENAL THRESHHOLD FOR GLUCOSE IN DIABETICS DURING PREGNANCY. Klin. Wchnschr. *34*:762-63, July 15, 1956.

The renal threshhold for glucose decreases during pregnancy in the diabetic as well as in the normal woman. The lowest threshhold levels are reached during the nineteenth and thirty-fourth weeks of gestation; accordingly, glycosuria increases during this period. During the post partum period, the threshhold rises and is usually higher than normal for about a half year or longer. It then returns to its original level.

Mosca, Leonardo (Dept. of Pathol., Cambridge Univ., England): CHANGES IN THE ISLETS OF LANGERHANS ASSOCIATED WITH AGE AND HIBERNATION. Quart. J. Exper. Physiol. *41*:433-41, October 1956.

With the use of modern staining technics, the ratio of alpha to beta cells for rats and bats is found to be at a high level in the young of each of these species. It falls to lower values at about the time of puberty because of an active mitotic multiplication of beta cells, demonstrable by means of colchicine. After the adult level of the alpha to beta-cell ratio has been reached, it is not affected by changes in the body state arising from hibernation.

Mulder, Donald W.; Bastron, James A.; and Lambert, Edward H. (Sects. on Neurol. and Physiol., Mayo Clin. and Mayo Foundation, Rochester, Minn.): HYPERINSULIN NEURONOPATHY. Neurology *6*:627-35, September 1956.

A syndrome of distal muscular atrophy and paresthesias associated with hyperinsulinism has been observed in twenty patients. The symptoms and findings in this syndrome are reviewed, and it is suggested that this syndrome is best described as "hyperinsulin neuronopathy." The differentiation of this syndrome from progressive muscular atrophy, peroneal muscular atrophy, and chronic neuronitis is discussed. It is suggested that hyperinsulin neuronopathy may account for some of the neuropathies seen in diabetic patients.

Murison, Paul J. (Dept. of Med., Ochsner Clin., New Orleans, La.): LONG RANGE COMPLICATIONS OF POORLY CONTROLLED DIABETES. J. Louisiana M. Soc. *108*: 137-41, April 1956.

Convincing evidence exists that degenerative complications associated with diabetes, including premature arteriosclerosis, retinitis, neuritis and intercapillary glomerulosclerosis, are caused to a large extent by biochemical and biophysical abnormalities, resulting from poor control of the disturbed glucose and lipid metabolism of diabetes. Clinical observations show that the incidence of these complications is appreciably lower in the patient who has best controlled his diabetes.

Olney, Mary B. (Univ. of California, Sch. of Med., San Francisco, Calif.): EDUCATION OF THE DIABETIC CHILD. Kaiser Foundation Med. Bull. *4*:251-56, July-August 1956.

Diabetes is an overwhelming calamity to the child in whom it is discovered and to all but exceptionally well-adjusted parents. A rigorous program of education must be instituted, if the patient and his family are to gain control of the situation. The primary aim of our program is to create in patient and family a realistic acceptance of certain facts regarding the patient's handicaps, his physical and psychologic needs, and his capacity to live fully. Instruction regarding diet presents one of the more difficult aspects of education of the diabetic child and his family. In this country, where immigrant groups have introduced less desirable foods from their cultures and have often adopted the less desirable from others and where many families skimp on food in order to buy cars or homes, carbohydrates form an undesirably high proportion of the diet. We teach that there is no food which the diabetic may not eat. How well he will be satisfied depends upon how much he knows about food. A common parental error is to translate into the child's dietary regimen the periodic episodes of self-indulgence for which the parents themselves feel a strong desire. The parent who accepts for himself the habit of the "Saturday night binge" is likely to feel that the child, too, should have a "free diet" on Saturday afternoon, interpreting this in a way that the child is permitted to eat ice cream, popcorn, soda pop, candy, or whatever comes to his mind. Children tend to give up this type of instability before many parents can be educated to do so. Another common error is that the parents' intense fear of insulin reactions may lead them to forbid all physical exercise. Many prefer that their child's urine specimen will always show a yellow to orange reaction rather than green to blue, in order to prevent an insulin reaction. The pediatrician, whose responsibility is to maintain the child in the best possible condition until he changes to the care of the internist, must counteract this tendency to lax control. Some children find it hard to recognize the aura of an insulin reaction. The author believes that it is useful for the child to undergo a number of minor reactions during hospitalization so

that he may learn to recognize this sensation.

Oppé, T. E.; Hsia, David Yi-Yung; and Gellis, Sydney S. (Dept. of Pediat., Harvard Med. Sch., Boston Lying-in Hosp., and Joslin Clin., Boston, Mass.): PREGNANCY IN THE DIABETIC MOTHER WITH NEPHRITIS. Lancet *1*:353-54, February 16, 1957

The authors review the course of forty-one class F diabetic pregnancies. The fetal wastage was 55 per cent. The infants were smaller than those usually delivered of diabetic mothers but showed the same tendency to hyaline membrane as the cause of neonatal death.

Oppenheimer, Henry E. (St. Louis Univ. Sch. of Med., St. Louis, Mo.): EARLY RECOGNITION OF DIABETES. J. Michigan M. Soc. *55*:1193-95, October 1956.

Methods have been described for the early recognition of diabetes, patterned after those used in diabetes detection. They include more critical laboratory studies than those usually performed. The advantages of early recognition of the disease are discussed. At its early discovery the aim is directed towards prevention of the complications of diabetes, both acute and chronic.

Rinzler, Seymour H.; Travell, Janet; Karp, Dorothy; and Charleson, Diana (Dept. of Pharmacol., Cornell Univ. Med. Coll., New York, N. Y.): DETECTION OF CORONARY ATHEROSCLEROSIS IN THE LIVING RABBIT BY THE ERGONOVINE STRESS TEST. Am. J. Physiol. *184*:605-12, March 1956.

Electrocardiograms were taken within fifteen minutes after administration of 0.05 mg./kg. and, on occasion, 1 mg./kg. of ergonovine maleate intravenously, for a total of ninety-four ergonovine stress tests on thirty-four rabbits. Fourteen animals served as controls, and twenty were cholesterol-fed. One series was kept on the diets for about two and one-half months and another for about five months. Positivity of the ergonovine stress test was indicated by a drug-induced depression of the S-T segment amounting to 0.5 mm. or more below the isoelectric level in either leads II or IV. Hearts were examined microscopically; aortas, grossly. In control animals, ergonovine stress tests were uniformly negative and coronary arteries and myocardium normal. In cholesterol-fed rabbits, the ergonovine stress test was likewise negative after two and one-half months, but there was atherosclerosis of the thoracic aorta, diffuse fatty infiltration of the myocardium, and marked cholesterolemia. After five months, the ergonovine stress test had become positive in all cholesterol-fed rabbits. At this time, each animal showed subendothelial lipid accumulation in the coronary arteries with occlusive intimal atherosclerosis and, in the myocardium, fatty infiltration and spotty hyaline degeneration. Data show a correlation of positivity of the ergonovine stress test with occlusive atherosclerosis of the small coronary arteries and myocardial damage. It is suggested that this test may provide an experimental procedure for the study of coronary atherosclerosis in the living animal.

Rippy, Edwin L. (Southwestern Med. Sch., Univ. of Texas, Dallas, Tex.): THE PREVENTION AND TREATMENT OF KETOSIS IN DIABETES. J. Louisiana M. Soc. *108*:185-90, June 1956.

No attempt has been made in this discussion to go into the complex biochemical and physiological alterations in diabetic ketosis, but rather, by intent, emphasis has been placed on clinical care which would tend to prevent this state and on the practical clinical application of the weapons we have for its treatment.

Shafer, Marion R. (Indianapolis, Ind.): DIABETES OF CHILDHOOD. J. Michigan M. Soc. *55*:1218-21, October 1956.

Every diabetic child requires insulin continuously from the day of diagnosis of the disease. Protamine zinc insulin finds its greatest usefulness in juvenile diabetes because of the unfailing occurrence of hyperglycemia during the night in the diabetic child. It is common knowledge, however, that good control is not possible with this or any of the present-day insulins alone. The preferred method is the use of separate injections of unmodified and protamine zinc insulins before breakfast and, frequently, unmodified insulin before supper. Except where supplemental unmodified insulin is used, experience with the use of the so-called intermediate-acting insulins, such as NPH and Lente, and insulin mixtures has been repeatedly disappointing in the child. The use of these has resulted in an increased instability with loss of carbohydrate tolerance. Such is the impression of others. The management of a juvenile diabetic patient resolves itself into just a careful day-by-day control of the condition as can be obtained. Compromises with ideal treatment must often be made because of practical consideration; however, these need not allow one to lose sight of the goal, which is to bring about physiologic conditions insofar as is possible. Only in this way can the degenerative complications be prevented and the diabetic child be enabled to live out a useful life span approaching that of the nondiabetic youngster.

Siegel, Benjamin V.; and Hughes, Ann M. (Virus Lab. and Radiation Lab., Univ. of California, Berkeley, Calif.): GLUCOSE OXIDATION BY NORMAL AND VIRUS-INFECTED MICE. Am. J. Physiol. *184*:356-58, February 1956.

The oxidation of uniformly labeled C^{14}-glucose by normal mice and mice infected with strain MEF poliomyelitis virus was determined in an apparatus designed to measure respiratory radioactivity excretion. The respiration of normal and virus-infected mice was found to be essentially the same over a ten-day period of infection, although the production of virus in the diseased animal during this time was considerable. It is concluded (a) that the rate and extent of viral multiplication in poliomyelitis is without apparent effect on the rate and extent of glucose metabolism by the infected intact host and (b) that variations in the rate and extent of glucose metabolism do not condition the temporal occurrence and gradation of clinical symptoms of infected animals.

Skouby, Arne P. (Medical Out-Patient Clin., University Hospital, Copenhagen, Denmark): CLINICAL ENDOCRINE DISTURBANCES IN DIABETICS; THEIR RELATION TO LATE DIABETIC LESIONS. Acta med. scandinav. *155*: 401-09, Oct. 15, 1956.

A series of 124 female and 101 male diabetics was examined for endocrine disturbances other than diabetes. Such definite endocrine disorders occurred in 23 per cent of the females and in 5 per cent of the males. Goiter was found in twelve women (10 per cent) and one man. Delayed menarche (after the age of seventeen) occurred in sixteen women (13 per cent) — before the onset of diabetes in eight of them. Growth disturbances occurred in four female and eight male patients. Four were small before the onset of the disease. One patient suffered also from irregular hyperplasia of the uterine mucosa and another from eunuchoidism. Therapy with chorionic gonadotropin from urine was followed by diabetes in three cases and by severe retinopathy in two diabetics. In patients with retarded growth, the incidence of late diabetic lesions was greater than expected.

Thomas, Wilbur A.; Lee, Kyu Taik; and Rabin, Erwin R. (Dept. of Pathol., Washington Univ. Sch. of Med., St. Louis, Mo.): FATAL ACUTE MYOCARDIAL INFARCTION IN DIABETIC PATIENTS. A COMPARATIVE STUDY OF NINETY-FOUR AUTOPSIED DIABETICS WITH MYOCARDIAL INFARCTION AND FOUR HUNDRED SIX AUTOPSIED NONDIABETICS WITH ACUTE MYOCARDIAL INFARCTION, WITH SPECIAL REFERENCE TO AGE AND SEX DISTRIBUTION. A. M. A. Arch. Int. Med. *98*:489-94, October 1956.

The authors have compared the age and sex distribution and various clinical and anatomical features of ninety-four diabetics who died of acute myocardial infarction (diabetic-myocardial infarction group) with the corresponding features of 406 nondiabetics who died of the same cause (nondiabetic-myocardial infarction group).

The average ages at death, in both sexes, are similar in the diabetic-myocardial infarction group to the corresponding ages in the nondiabetic-myocardial group.

In previous studies, they have demonstrated that, prior to 1940, acute myocardial infarction in the Barnes Hospital autopsy series was significantly more common among men than it was among women but that since 1940 the incidence of acute myocardial infarction among women has been similar to that among men. The entire autopsy series was divided into diabetic and nondiabetic men and women, and the incidence of acute myocardial infarction was determined for each sex in each group (diabetic and nondiabetic) and for each period (before and after 1940). The results obtained indicate that, in this autopsy series since 1940, acute myocardial infarction is significantly more common among diabetic women than among diabetic men; whereas the incidence is still significantly greater among nondiabetic men than among nondiabetic women.

Other clinical and anatomical characteristics of diabetics and nondiabetics with acute myocardial infarction are compared in this study, and most characteristics are found to be similar.

Unger, Robert H.; and Davidson, Joseph W., Jr. (Univ. of Texas, Southwestern Med. Sch., and Veterans Administration Hosp., Dallas, Tex.): CURRENT STATUS OF ARYL SULFONYLUREAS IN TREATMENT OF DIABETES MELLITUS. J. A. M. A. *162*:447-53, Sept. 29, 1956.

A study was made of the effect of tolbutamide (Orinase) therapy in eighteen diabetics. Daily oral administration of tolbutamide in doses of 3 to 6 gm. per day suppressed glycosuria and diminished fasting hyperglycemia in seven of twelve patients with stable adult diabetes. Six of the seven diabetics who responded to treatment had conditions that were mild enough to be adequately controlled by diet alone. In only one case had insulin therapy been necessary for adequate control of the disease. Large doses of tolbutamide did not benefit patients with labile diabetes and caused hypoglycemic shock in one patient. One patient with diabetes provoked by steroid therapy did not benefit from tolbutamide.

Walker, Joan B. (Leicester, England): NEUROLOGICAL MANIFESTATIONS OF HYPOGLYCAEMIA. Brit. M. J. *1*:165, Jan. 19, 1957.

The author writes about her experience in observing patients with various neurological manifestations of hypoglycemia.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1957-1958

HONORARY PRESIDENT, ELLIOTT P. JOSLIN, M.D., *Boston*

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JOHN A. REED, M.D., *Washington, D.C.*

FIRST VICE PRESIDENT
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WILLIAM H. OLMSTED, M.D., *St. Louis*

EXECUTIVE DIRECTOR
J. RICHARD CONNELLY, *New York*

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ARTHUR R. COLWELL, SR., M.D., *Chicago*
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THOMAS P. SHARKEY, M.D., *Dayton*
JOHN H. WARVEL, SR., M.D., *Indianapolis*
ROBERT H. WILLIAMS, M.D., *Seattle*

TERM EXPIRING 1959

LOUIS K. ALPERT, M.D., *Washington, D.C.*
W. WALLACE DYER, M.D., *Philadelphia*
EDWIN W. GATES, M.D., *Niagara Falls*
HARVEY C. KNOWLES, JR., M.D., *Cincinnati*
ARNOLD LAZAROW, M.D., *Minneapolis*
E. PAUL SHERIDAN, M.D., *Denver*

TERM EXPIRING 1960

CHARLES H. BEST, M.D., *Toronto*
THADDEUS S. DANOWSKI, M.D., *Pittsburgh*
EDMOND K. DOAK, M.D., *Houston*
GARFIELD G. DUNCAN, M.D., *Philadelphia*
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LEON S. SMELO, M.D., *Birmingham*

PAST PRESIDENTS

HENRY B. MULHOLLAND, M.D., *Charlottesville, Va.;*
HENRY T. RICKETTS, M.D., *Chicago;* FREDERICK W. WILLIAMS, M.D., *New York*

The Seventeenth Annual Meeting

The Seventeenth Annual Meeting of the American Diabetes Association was held in New York City, June 1-2, 1957. At the Business Session on June 2, John A. Reed, M.D., Washington, D. C., was elected President for this organizational year to succeed Frederick W. Williams, M.D., New York. Alexander Marble, M.D., Boston, was elected First Vice President, and Francis D. W. Lukens, M.D., Philadelphia, Second Vice President. Franklin B. Peck, Sr., M.D., Indianapolis, was re-elected Secretary and William H. Olmsted, M.D., St. Louis, Treasurer. Drs. Reed and Marble served in 1956-57 as First and Second Vice Presidents respectively. All the officers will serve through the Eighteenth Annual Meeting of the Association, which will be held in San Francisco, June 21-22, 1958.

At this Business Session Charles H. Best, M.D., Toronto, Thaddeus S. Danowski, M.D., Pittsburgh, Edmond K. Doak, M.D., Houston, Garfield G. Duncan, M.D., Philadelphia, Henry E. Marks, M.D., New York, and Leon S. Smelo, M.D., Birmingham, were elected to the Council for the three-year term ending in 1960. Drs. Best and Duncan were re-elected to the Council.

Registration for the Annual Meeting established an all-time high as reflected in the following comparisons.

	Active Members	Guest physicians	Other guests	Total
1957				
New York	493	182	43	718
1956				
Chicago	406	130	29	565
1955				
Atlantic City	408	137	35	580
1954				
San Francisco	199	99	16	314
1953				
New York	438	198	7	643
1952				
Chicago	325	102	5	432
1951				
Atlantic City	342	125	10	477
1950				
San Francisco	162	83	6	251

Thirty-two meetings of Committees of the American Diabetes Association and meetings of the Board of State Governors and Assembly of Delegates were held between May 27 and June 3. The report of the Annual Business Meeting will be published in the September-October issue of DIABETES.

Featured for the first time at an Annual Meeting was an exhibit of ADA printed material and a display of materials issued by Affiliate Associations.

THE SCIENTIFIC SESSIONS

Sixteen scientific papers were presented during the Scientific Sessions, and eighteen were read by title. Two panel discussions, "Pregnancy" and "Oral Hypoglycemic Agents," were held. Participating in the first were Lester J. Palmer, M.D., Moderator, William P. Given, M.D., (by invitation), Edmund L. Shlevin, M.D., Priscilla White, M.D., and Hugh L. C. Wilkerson, M.D. The second panel included Arthur R. Colwell, Sr., M.D., Moderator, Henry Dolger, M.D., Garfield G. Duncan, M.D., Rachmiel Levine, M.D., and Mary A. Root, Ph.D.

The Banting Memorial Lecture was delivered at the Scientific Session on June 1 by DeWitt Stetten, Jr., M.D., Ph.D., Associate Director in Charge of Research, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland. The Lecture, entitled "Certain Aspects of the Metabolism of Glycogen," will be published in the September-October issue of DIABETES.

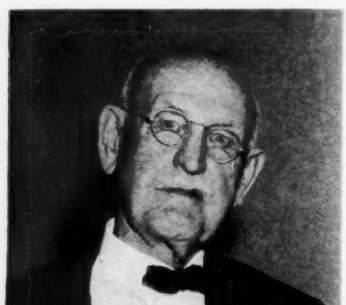
THE ANNUAL BANQUET

The Annual Banquet was held Saturday evening, June 1, with 335 persons in attendance. Dr. Louis H. Bauer, Secretary General of the World Medical Association and a former President of the American Medical Association, delivered an address on the subject, "Medical Freedom—National and International." Frederick W. Williams, M.D., President of the American Diabetes Association, spoke on "Whence, Where, Whither."

During impressive ceremonies, Dr. Williams presented Banting Medals to DeWitt Stetten, Jr., M.D., Ph.D., the Banting Memorial Lecturer, and John R. Murlin, Ph.D., D.Sc., Honorary Professor Emeritus, Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York. Dr. Williams was himself presented with a Banting Medal by Henry T. Ricketts, M.D., immediate Past President of the American Diabetes Association. The pictures on these pages were taken at the Banquet.

DR. RICKETTS (LEFT) PRESENTS BANTING MEDAL TO DR. WILLIAMS

The first Lilly Award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal, was presented by Dr. Williams to Solomon A. Berson, M.D., Chief, Radioisotope Service, Veterans Administration



DR. MURLIN

DR. STETTEN

DR. BERSON

Hospital, Bronx, New York. The award is given in recognition of demonstrated research in the field of diabetes.

Special citations were awarded to outstanding laymen. A joint citation, awarded for the first time, was presented to Mr. and Mrs. C. Paul Tiley, of Ferguson, Missouri. They received their award "in recognition of their devotion to the well-being of diabetics in the City of St. Louis and their outstanding efforts in the establishment of camping facilities for the diabetic children in that area."

Merton Koplin, noted television and radio producer, was awarded a special citation "in grateful recognition of his generous and unselfish support of the nationwide public diabetes education and detection program through his valuable and outstanding services in the field of television and radio as advisor, director and producer."

SIXTH POSTGRADUATE COURSE

As previously announced, the American Diabetes Association will hold its Sixth Postgraduate Course in Diabetes and Basic Metabolic Problems Jan. 22, 23 and 24, 1958, in Atlanta, Georgia. Christopher J. McLoughlin, M.D., Atlanta, will serve as Director, with C. Raymond Arp, M.D., and Walter L. Bloom, M.D., Atlanta, as Associate Directors. This will be the first meeting of the Association to be held in the Southeast. Members and others who expect to attend may also wish to take advantage of this Georgia location in planning their winter vacations.

The preliminary program will be sent to the members of the Association in the near future. The Atlanta Biltmore will serve as headquarters hotel and registrants for the Course will be furnished with reservation cards. Those who plan to attend the Course are urged to register as soon as possible. Fees are $40 for members and $75 for nonmembers of the Association. All inquiries and applications should be addressed to the national office of the American Diabetes Association.

EIGHTEENTH ANNUAL MEETING

The American Diabetes Association will hold its Eighteenth Annual Meeting in San Francisco June 21-22, 1958, immediately prior to the Annual Session of the American Medical Association, June 23-27. Information about hotel arrangements will be announced shortly.

SCIENTIFIC PROGRAM

Physicians and other scientists are invited by Francis D. W. Lukens, M.D., Chairman of the Committee on Scientific Programs, to submit abstracts of papers which they would like to present at the Scientific Sessions. Those interested are requested to submit eleven copies of the abstracts to expedite review by the Committee. They should be sent to Dr. Lukens in care of the national office.

1956-57 MEDICAL STUDENT-INTERN ESSAY CONTEST AWARDS

The Committee on Scientific Awards of the American Diabetes Association takes great pleasure in announcing the selection of Richard J. Feinberg, M.D., Philadelphia, as winner of the fifth (1956-57) Medical Student-Intern Essay Contest for the best paper reporting original work in the field of diabetes by medical students and interns, whether laboratory investigation or clinical observation. Among papers reviewed by the Committee, "An Immunological Method for the Assay of the Serum Level of Insulin," by Dr. Feinberg, was chosen as the outstanding contribution. He received an award of $250, made possible through the kindness of the St. Louis Diabetes Association.

SAMI A. HASHIM, M.D.

RICHARD J. FEINBERG, M.D.

Richard Feinberg was born in Fall River, Massachusetts, in 1922, and attended the Fall River Public Schools and Wilbraham Academy. A graduate of Wesleyan University in Middletown, Connecticut, with a B.A. degree in 1945, he received an Sc.M. degree in 1948 in Bacteriology from Brown University, Providence, Rhode Island. In June 1957 he received both an M.D. and a Ph.D. degree from the University of Pennsylvania. His thesis for the latter degree was entitled "Some Characteristics of Pollen Antigens Adsorbed onto Erythrocytes." Dr. Feinberg is at present serving an internship

at the University of Illinois Research and Educational Hospitals, Chicago.

An award of $50 for the best review article or case report was given to Sami A. Hashim, M.D., Boston, for "Acute Necrotizing Renal Papillitis in a Diabetic Treated by Nephrectomy and Complicated by Waterhouse-Friderichsen Syndrome." Dr. Hashim was born in Damour, Lebanon. He received a B.A. degree in 1950 from the American University of Beirut, and an M.S. degree from the same university in 1952 after two years of research work in the histochemistry of polysaccharides and nucleic acids. After receiving his M.D. degree in 1955 from the University of Buffalo School of Medicine, Buffalo, New York, he served an internship at Peter Bent Brigham Hospital in 1955-56 and was Assistant Resident in 1956-57. Dr. Hashim is at present a Research Associate at the Harvard School of Public Health, working on the metabolism of atherosclerosis. He is also Assistant in Medicine at Peter Bent Brigham Hospital.

Excellent papers were submitted in this year's competition with honorable mention given to two additional papers, whose authors were awarded one-year subscriptions to DIABETES. Information about the authors and their papers follows: James R. Fitzgerald, M.D., Hartford, Connecticut, submitted a paper entitled "A Study in Nephrosis of Serum Lipids Including the Nonesterified Fatty Acids." Dr. Fitzgerald received a B.A. degree from the University of Connecticut in 1953 and an M.D. degree from Yale University in June 1957. He is presently serving a rotating internship at St. Francis Hospital in Hartford. Maynard H. Makman, Euclid, Ohio, also won honorable mention for his paper, "Studies on Some Actions of Sulfonylurea Derivatives." Makman, who received a B.A. degree from Cornell University in 1955, has completed two years at Western Reserve University School of Medicine. At present, he is a candidate for both the M.D. degree and the Ph.D. degree in Pharmacology, and has an appointment this summer as a Research Fellow in Pharmacology.

1957-58 MEDICAL STUDENT-INTERN ESSAY CONTEST

The sixth Medical Student-Intern Essay Contest, sponsored by the American Diabetes Association, is open to medical students, interns and physicians within two years after their graduation from medical school. Any subject relating to diabetes and basic metabolic problems may be selected.

An award of $250 is offered to the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. This prize is again made possible through the kindness of the St. Louis Diabetes Association. An award of $50 will be given for the best review article or case report. Papers will be judged on the basis of value of the material and method of presentation.

Members of the American Diabetes Association and subscribers to DIABETES are requested to encourage medical students and interns to enter the contest. Entrants should submit the original and two copies of their manuscript. The manuscript should be typewritten and double-spaced, and mailed by April 1, 1958 to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

1958 LILLY AWARD

The following stipulations govern the contest for the second annual Lilly Award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal. The Award will be given at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco, June 21-22, 1958.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training, and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be an-

nounced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on the subject of his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

RESEARCH FELLOWSHIPS

As previously announced, the Committee on Research and Fellowships of the American Diabetes Association is planning to award at least one Fellowship for the 1958-59 academic year (July 1, 1958-June 30, 1959). The deadline for applications is Nov. 15, 1957. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

Correction: The announcement in the May-June 1957 issue of DIABETES concerning the three Research Fellowships awarded for the 1957-58 academic year should have stated that Dr. Jefferson Earle White, Jr., will work with Frank L. Engel, M.D., Professor of Medicine, Assistant Professor of Physiology, Director, Division of Endocrinology, Duke University School of Medicine, Durham, North Carolina.

THIRD CONGRESS, INTERNATIONAL DIABETES FEDERATION

As previously announced, the Third Congress of the International Diabetes Federation will be held in Düsseldorf, Germany, July 21-25, 1958. Professor Dr. K. Oberdisse, Düsseldorf, has been designated Chairman and Priv. Doz. Dr. K. Jahnke, Düsseldorf, Organizing Secretary, of the Congress. Additional information about the Congress will be published in future issues of this Journal.

DIABETES WEEK, 1957

The week of November 17-23 will be observed as Diabetes Week. Each year the week which precedes Thanksgiving is the official Diabetes Week as selected by the Council upon recommendation of the Executive Committee. Affiliate Associations and Committees on Detection of County and State Medical Societies are urged to note these dates in making plans for development of Public Education and Detection programs. This year's Diabetes Week poster is reproduced on page A-7.

NEW MEMBERS

ACTIVE

The following were elected as of June 1, 1957:

California	
Frye, John W.	Fresno
Ryan, William H.	Modesto
Florida	
Marcus, Nathan L.	Tampa
Minnesota	
Senders, Wilbur L.	Rochester
New Mexico	
Massey, Robert U.	Albuquerque
New York	
Wolff, John L. E.	New York
North Carolina	
Amatruda, Thomas T., Jr.	Durham
South Dakota	
Sanderson, Everett W.	Sioux Falls

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION will sponsor a Symposium entitled "Recent Developments in Diabetes Mellitus (Pathology, Diagnosis and Therapy)" on Wednesday, November 20, in the Walton Room of the Drake Hotel, with Henry T. Ricketts, M.D., as moderator. A fee of $25.00 will be asked of all registrants for the course, with the exception of members of the Chicago Diabetes Association or the American Diabetes Association, who may register without charge. Enrollment is limited to 200. The following program will be given:

9:00-9:30 a.m. "Pathology of Diabetes Mellitus in Man," Aaron Arkin, M.D. 9:40-10:10 a.m.: "Metabolic Derangements in Diabetes Mellitus," Rachmiel Levine, M.D. 10:20-10:50 a.m.: "The Metabolism of I^{131}-labeled Insulin," Solomon A. Berson, M.D., and Rosalyn S. Yalow, Ph.D., New York. 11:00-11:30 a.m.: "Insulin Resistance," Arthur R. Colwell, Sr., M.D.

A Question and Answer Period will be held during the luncheon recess.

1:30-2:00 p.m.: "Oral Hypoglycemic Agents in the Treatment of Diabetes," Alexander Marble, M.D., Boston. 2:10-2:40 p.m.: "Current Concepts of the Pathogenesis of Diabetic Retinopathy," David A. Rosen, M.D., Kingston, Ontario. 2:50-3:20 p.m.: "Diabetic Retinopathy," Robert M. Kark, M.D. 3:30-4:00 p.m.: "Juvenile Diabetes," Howard S. Traisman, M.D.

The NEW YORK DIABETES ASSOCIATION (Clinical Society) will present its Fifth Annual Symposium Day on Diabetes Mellitus on Saturday, October 12, in New York City at the Hunter College Playhouse Auditorium. The Symposium is entitled "Fat and Diabetes."

The following preliminary program has been arranged: "Ketogenesis," Dr. William C. Stadie, University of Pennsylvania; "Glycolytic Pathways and their Relation to Synthesis of Cholesterol and Fatty Acids," Dr. Marvin Siperstein, University of Texas, Southwestern Medical School; "An Abnormality of Nonesterified Fatty Acid Metabolism in Diabetes Mellitus," Dr. Vincent Dole, Rockefeller Institute for Medical Research; "Serum Triglycerides in Relation to Carbohydrate Metabolism in Health and Diabetes," Dr. Margaret J. Albrink and Dr. Evelyn Man, Yale University School of Medicine; "Relation of Dietary Protein to Transport and Deposit of Fat," Dr. Robert Olson, University of Pittsburgh; "Action of Insulin on Adipose Tissue," Dr. F. X. Hausberger, Jefferson Medical College; "Abnormal Fat Transport," Dr. Stanley Hartroft, Washington University; "Obesity, Fat Metabolism and Diabetes," Dr. David Adlersberg, Columbia University College of Physicians and Surgeons.

Physicians, medical students and other members of the profession are invited to attend. While there is no attendance fee, advance registration is required for admittance. For information write to the New York Diabetes Association, 104 E. 40th St., New York 16, N.Y.

NEWS NOTES

ADA MEMBERS PARTICIPATE IN AMERICAN COLLEGE OF PHYSICIANS MEETING

Among members of the American Diabetes Association who took part in the Thirty-Eighth Annual Session of the American College of Physicians in Boston, April 8-12 were: Richard P. Stetson, M.D., General Chairman and Chairman of the Committee on Arrangements, and Robert F. Bradley, Jr., M.D., Reed Harwood, M.D., Alexander Marble, M.D., and James H. Townsend, M.D., members of the Committee; Reed Harwood, M.D., Chairman of the Committee on Auditoria and Equipment, and L. Tillman McDaniel, M.D., and Carey M. Peters, M.D., members of the Committee; Arthur D. Baldwin, M.D., Eugene F. McAuliffe, M.D., members of the Committee on Public Relations and Hospitality; Frank N. Allan, M.D., and Hugh L. C. Wilkerson, M.D., members of the Committee on Scientific Publicity; Garfield G. Duncan, M.D., member of the Committee on Technical Exhibits; Alexander Marble, M.D., Chairman of the Committee on Televised Hospital Clinics, and C. Cabell Bailey, M.D., George W. Dana, M.D., and Leo P. Krall, M.D., members of the Committee; James H. Townsend, M.D., Chairman of the Committee on Transportation, Robert F. Bradley, Jr., M.D., Co-Chairman, and William McFarland, M.D., Committee member.

Howard F. Root, M.D., President of the Massachusetts Medical Society, gave one of the Addresses of Welcome. Nineteen members of the American Diabetes Association took part in the Scientific Program.

ANNUAL MEETING OF THE AMERICAN DIETETIC ASSOCIATION

The 40th Annual Meeting of The American Dietetic Association will be held October 22 through October 25 at Miami, Florida. Sessions and exhibits will be held at the Dinner Key Auditorium. The headquarters hotels will be The McAllister and The Columbus. The meeting will bring to the membership the latest developments in the sciences of nutrition, diet therapy, food technology, education and food administration.

PERSONALS

CHARLES ALEXANDER BEST, son of Dr. and Mrs. Charles H. Best, who at the age of 25 is already a recognized botanist, has been elected to the Canadian Parliament. He carried his district (Halton County) by the largest majority in its history to become one of the youngest members of the House of Commons.

OBITUARY

LAWRENCE GREENMAN, M.D., Pittsburgh, died last year at the age of 35. Dr. Greenman was born in Brooklyn, New York, and received his premedical education at New York University. He then attended Yale University School of Medicine and was graduated in 1944. After internship in Medicine at New Haven Hospital, he became successively Assistant Resident in Medicine at Barnes Hospital, St. Louis, Missouri, Assistant Resident in Medicine at New Haven Hospital and Associate Resident in Medicine at New Haven Hospital. Dr. Greenman was Assistant Professor of Research Medicine at the University of Pittsburgh School of Medicine since 1949, an Associate Staff Member at Presbyterian-Woman's Hospital of Pittsburgh, Associate Physician at Renziehausen Memorial Ward and Clinic and Staff Physician at Falk Clinic. A member of the American Diabetes Association since 1948, he was also a member of the Clinical Society of the Pittsburgh Diabetes Association and served as second Vice-President during 1952-53.